CLINICAL SCIENCE

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HEART

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VOL 5.

Sir THOMAS LEWIS.

It is with deep regret that we record the death of Sir Thomas Lewis on March 17th, 1945, in his 63rd year. Lewis edited this journal from its first appearance as Heart in 1908, he was instrumental in its change of name and scope in 1933, and continued as editor until the Autumn of 1944 when he felt that a younger man should take his place A fuller appreciation will appear in the next issue.

AUTHORS

CONTENTS.

A SIMPLE STANDARD PROCEDURE FOR OBTAINING BLOOD HAVING A HÆMOGLOBIN CONTENT IDENTICAL WITH ARTERIAL BLOOD

By E B REEVE and N A NEVILLE †

(*From the Clinical Research Unit, Guy's Hospital*)

SINCE arterial blood comprises the mixed venous bloods after their passage through the pulmonary circulation, its hæmoglobin content indicates the balance of loss and gain of fluid and pigment by the blood of the whole body at the time at which it was drawn   It is therefore for many purposes the blood best suited to hæmoglobin estimations , for local causes, natural or artificial, cannot alter the arterial to the same extent as the venous hæmoglobin content   In a series of experiments we required frequent knowledge of the arterial hæmoglobin content * Repeated arterial punctures, though under the proper circumstances neither painful nor difficult, require time and considerable care   We therefore developed a simple standard procedure, which we have found can be relied upon to yield blood not differing in its hæmoglobin content from arterial blood taken within a short interval by more than the errors of the methods of estimation and blood handling used

The method is as follows   The left or right hand, forearm and elbow of the experimental subject are immersed in water in an arm bath or sink maintained at 40 to 42°C for ten to fifteen minutes   This results in marked dilatation of the vessels of the hand and a rapid blood flow through them When there is evidence of marked vasodilatation the hand and forearm are removed from the bath, dried and covered with a towel to diminish heat loss, and blood is withdrawn from a vein at or a little above the wrist, usually and conveniently one of the veins running along the radial aspect of the wrist   While blood is being withdrawn the subject lays one finger

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† Work undertaken on behalf of the Medical Council

* It is frequently stated that under certain conditions for example, absence of stasis and warmth of extremity, the hæmoglobin contents of arterial, skin prick and venous bloods are identical but we have been unable to find good evidence for this in past records   We have found no series of estimations on man using accurate methods of hæmoglobin estimation such as here reported

a few centimetres above the point of venepuncture with just sufficient weight to prevent the vein collapsing during the withdrawal of blood During the warming the forearm is flexed at the elbow and has generally not hung more than a few centimetres below the level of the apex beat

In the table (Section A) the hæmoglobin content of 18 samples (1 to 3 c c) of blood drawn as described from 12 adult males, is compared with the hæmoglobin content of blood samples (1 to 3 c c) drawn from the radial artery of the same side either just before or just after venepuncture   The hæmoglobin content was estimated by a photo-electric method (3)   Blood was either pipetted immediately on drawing, when no anticoagulant was used, or coagulation was prevented by solid heparin   All estimations

TABLE

		Section A						Section B			
Subject	Temperature of water	Arterial hæmoglobin	Venous hæmoglobin	Difference	Interval between withdrawal of art and venous bloods	Subject	Temperature of water	Arterial hæmoglobin	Venous hæmoglobin	Difference	Interval between withdrawal of art and venous bloods
	°C	%	%	%	Mins		°C	%	%	%	Mins
E B R	40	94 4	94 8	+0 4	3	N A N	44	95 1	95 3	+0 2	10
"	40	93 3	93 3	0 0	6	"	44	100 7	100 8	+0 1	13
"	41	93 0	92 8	—0 2	3	J S S	44	101 2	104 0	+2 8	8
N A N	40	96 6	97 0	+0 4	6	E B F	44	97 9	99 2	+1 3	13
"	40	97 5	96 6	+0 9	5	T S C	44	102 0	103 6	+1 0	8
"	41	99 5	98 9	—0 6	10	H R.H	44	98 6	100 5	+1 9	8
D S	40	109 8	109 3	—0 5	3	H A T	44	103 0	100 4	—2 6	8
H K	37	110 4	110 4	0 0	15	H K	44	105 5	107 0	+1 5	8
H A T	40	94 0	94 8	+0 8	2						
J	41	91 7	91 7	0 0	2						
R	41	100 5	98 3	—2 2	2						
R T G	40 42	103 6	103 4	—0 2	3						
	41	100 7	100 8	+0 1	3						
F J P	41	97 1	96 5	—0 6	5						
E D B	40 42	101 7	102 4	+0 7	3						
"	41	103 6	103 7	+0 1	2						
"	41	97 7	98 2	+0 5	10						
T "	41	94 7	94 9	+0 2	5						

were made in duplicate and precautions were taken to minimize R B C sedimentation   Under the circumstances of this experiment a difference of 1% hæmoglobin is significant and indicates a difference of pigment due either to errors in technique, for example sedimentation or inaccuracies in pipetting, or to a significant difference in vascular hæmoglobin content   The duplicate determinations (not given) in only one estimation differed by more than 1%, in the majority by 0 7% or less, so that errors due to sedimentation and inaccuracies in pipetting can be neglected

Two practical points require mentioning The hand and forearm should not be warmed above 42°C At 44°C, though there is greater vasodilatation, the results given in the table, Section B, suggest that there is a significant slight increase of venous compared with arterial hæmoglobin content It should be noted, however, that these were our earliest experiments when we had less experience of arterial puncture The vasodilatation caused by warming, which in subjects comfortably warm is great, is less if to start with there is much peripheral vasoconstriction, as when the subjects are cold These observations have been made on subjects some comfortably warm, some chilly, but none markedly cold

The reasons for our choice of this method are as follows Under conditions of marked vasodilatation there is a very rapid blood flow through the hand, namely, up to 30 c c per 100 c c hand tissue per minute (2) Much of this blood passes through the widely dilated arterio-venous anastomoses The extent of vasodilatation is nicely observed from the colour of the hand and the rapidity of filling of the hand veins when, after emptying by raising them above heart level, they are congested The hand is placed in a constant and controlled environment Blood is better withdrawn from the wrist or distal forearm veins than from veins at the back of the hand The former are thicker walled and stand frequent puncturing Further, in many subjects repeated punctures at the wrist cause little pain We preferred the forearm veins to the antecubital veins for the reason that blood from the antecubital veins comes from two main sources, the skin and subcutaneous tissues of the hand and forearm, and the muscle mass of the forearm, which under varying conditions vary their contributions, while under the conditions described, blood from the wrist veins comes mainly from the skin and subcutaneous tissues of the hand It is worth noting that active muscles form considerable quantities of lymph, and Barcroft (1) has shown them to yield a more concentrated venous than arterial hæmoglobin

REFERENCES

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(2) GRANT AND PEARSON Clinical Science, 1938, **3**, 119

(3) REEVE (To be published)

OBSERVATIONS UPON A VASCULAR AXON REFLEX A CORRECTION

By THOMAS LEWIS *

(Department of Clinical Research, University College Hospital Medical School)

IN a paper (2) published in the last number of this journal a curious case of urticaria was described in which a constellation of wheals was produced around a central and quite local stimulus   This reaction was shown to depend upon the functional integrity of cutaneous nerves, and upon an axon reflex in them

Evidence was also produced which seemed to show the nerves in question to be cholinergic   Among other evidence it was stated that the skin of the patient reacted unusually to acetylcholine (carbachol)   To a given dose ionised into the forearm, full redness of the skin and a crop of local wheals appeared   The same dose introduced into 3 control subjects gave very slight reddening and no wheals

Recently I had occasion to test the skin of two other patients, in whom it was thought possible that a similar susceptibility to acetylcholine might be found and these skins both gave positive reactions   The original patient was a woman of 26 years , the controls to that case were two young and one older man   As the second and third patients were also young women I thought it wise to test the skin of a number of young women and was surprised to find that, without exception, these all gave positive results A fresh series of young men was tested simultaneously and these almost without exception gave negative results like the first controls   The whole series of controls is given in the accompanying table   In the original patient 0 1% carbachol was used, 40 microamps being passed from 10 volt accumulators for 10 minutes   This gave the whealing described   10 microamps gave local reddening and a few wheals

In the following controls the full current used was 37 microamps to compensate for a slightly smaller anode (19mm diam) than that used in the original case , otherwise the conditions were unchanged   In most of

those that reacted a current of 10 microamps was subsequently tested
0 1% carbachol was used in each case and the current passed for 10 mins

	Subject	Age	Microamps	Reactions of anodal skin
Females	C	22	37	Covered with small wheals
			10	Redness and a few small wheals
	B	22	37	Covered with small wheals
	J	21	37	Almost confluent wheals
	-		10	Redness and a few small wheals
	D	22	37	Confluent wheals
			10	Redness and a few wheals
	W	23	37	Many small wheals
			10	Redness and a few wheals
	R	29	37	Many small wheals
	H	34	37	A small crop of wheals
Males	H	32	40	Slight local redness
	T	26	40	Slight local redness
	L	61	40	Slight local redness
	M	22	37	Local redness only
	W	22	37	Local redness and doubtful whealing
			10	Local redness
	D	20	37	A little redness
	B	22	37	Local redness
	B	24	37	Local redness
	C	23	37	Many small wheals
			10	Redness and a few small wheals
	N	22	37	Redness and a few small wheals
	E	22	37	Patchy redness
	H	26	37	Patchy redness, doubtful whealing

Thus while 7 female subjects all gave whealing, out of 12 males only
one gave a comparable reaction No reason for this unexpected difference
between female and male is known The new control tests weaken the
evidence for the conclusion of my earlier paper that the nerves concerned
in the axon reflex action there described are cholinergic , they also weaken
the similar evidence put forward by Grant, Pearson and Comeau (1) in the
paper in which they regard the nerves which they found involved in reflex
urticaria to be cholinergic These workers used " doryl " to test the skin
by ionisation and found their control subjects negative , but here again
the patients were with one exception female and the controls, as Dr Grant
has since informed me, were males I have since ionised doryl (0 05%)
into the skin of two young women, using the same current density as that
of the original observations, and in both instances obtained crops of wheals
though less prominently than in the carbachol experiments and without
the surrounding flares of the original doryl experiments

Though the evidence put forward for the cholinergic nature of the
nerves involved in the urticarias recorded is weakened, it has not gone
In the cases by Grant and his associates, and in my own patient, pilocarpine
injected subcutaneously gave an intense urticarial rash, a reaction that
does not occur in controls of either sex , and atropine similarly administered
interfered with the usual urticarial reactions In the cases of Grant and his
associates too, the local action of doryl seems to have been more emphatic
than in both their controls and my own special controls in young women

Summary

1   For a reason unknown, the skin of young women appears to be more susceptible to the action of cholin derivatives, ionised into them, than does that of young men

2   This fact is relevant to observations made upon the nervous mechanism involved in urticaria of reflex origin, and to the conclusions previously drawn from them   For the conclusion that the nerves involved are cholinergic depended in part upon relative insusceptibility of control skin to choline derivatives, and this would not have been accepted as so clearly demonstrated had the control skins been those of female instead of male subjects

PEFERENCES

(1) GRANT PEARSON AND COMEAU   Clinical Science, 1936, 2, 253

(2) LEWIS   Clinical Science, 1942 4 365

NOTE —The control subject (C in the table) has since shown heat urticaria which prompts the suggestion that females are in general more predisposed to the relevant urticaria from heat than males

EFFECTS OF SUPERCOOLING SKIN

By THOMAS LEWIS *

(Department of Clinical Research, University College Hospital Medical School)

ALTHOUGH recorded observations on freezing animal tissues are numerous, records of their supercooling are infrequent One of the earliest is by Kodis (1), who in attempting to fix the freezing point cooled frog's muscle as low as —18° without freezing it He cooled a live frog to —10° without changing its reaction to stimuli when it was rewarmed

In a paper written with Love in 1926 (3), I described the phenomenon of supercooling of human skin, giving one instance in which the temperature of the skin was reduced on its surface to —20° and in its deeper layers to as low a point as —9°, without freezing occurring It was said by us that whealing was occasionally seen as an after-effect of surface cooling to about —20° Particular attention was given to the exclusion of freezing in these observations, but as whealing was seen in one subject only and that but rarely, we were unable to investigate the matter as fully as seemed desirable Recently, however, a reliable method of whealing the skin by supercooling has been found and it is these observations that are now to be described

The difficulty has been to produce an adequate degree of cooling over a sufficient period of time without actual freezing Reducing the surface temperature to —15° for periods of 5 or even 10 mm have not been seen to provoke subsequent whealing, —15° for 15 mm gives whealing quite exceptionally, and surface temperatures below this level have hitherto nearly always given freezing within a few seconds

In the present observations the skin used has been the middle of the front of the forearm It has been cooled by means of the apparatus previously described, namely, a bar of copper 1 5 cm square in section One end of this is immersed in acetone cooled by adding CO_2 snow to it, and the other is applied to the skin The temperature of the surface of this copper bar, beforehand and while in contact with the skin, is read from a thermal junction attached to the surface of the metal Now when this end of the bar is exposed to the air of the room, it very quickly becomes coated with very fine crystals of ice It was in this frost covered state that

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the bar was applied to the skin in our earlier observations, and it was probably owing to this hoar frost that supercooling could not be carried to its lowest points. It was recognised in those observations that soaking the skin in water predisposed to freezing and that careful drying and greasing the skin assisted supercooling. The only difference in the present procedure has been to keep the surface of the metal free of frost, which is readily done by brushing cold acetone over the metal surface before applying it to the skin, or, alternatively, lifting the copper bar directly out of acetone cooled to the desired point and applying it at once to the skin. If this procedure is followed, little difficulty is experienced in maintaining metal, cooled to —20° or —22° or even —25° on the skin for 10 seconds, and at the higher temperatures for much longer periods, without freezing happening.

As previously described, if freezing happens it cannot be overlooked, as soon as it starts there is a sensation of pricking or stinging at the site of contact and, if the bar is raised on the instant, a thin scale of frozen skin is always found, if contact is maintained at the stated temperatures this scale rapidly thickens and hardens until it becomes a hard dense plaque within a few seconds. The results now to be described as the result of supercooling are from applications of the bar giving no trace of these sensations, and in which on raising the bar at the end of appropriate but varying times, the skin is normal in appearance, lacking the opacity of frozen skin, and being quite supple. It is to be emphasised that there is no difficulty in being sure that freezing is not concerned, supercooling and freezing give different immediate effects and, as will be seen, they give different after-effects.

When the metal bar at —21° is brought into close contact with the skin, the temperature of its face rises about 1° very quickly, its temperature then becomes steady or almost so, rising perhaps another 0 5° in a minute. Thus the actual surface of skin originally warm falls almost at once to —20°. These facts are ascertained by recording the temperatures of the face of the bar from first to last. In these, as in the earlier observations, a thermal junction has sometimes been introduced directly beneath the skin to sample deep temperatures. Such a junction shows that when the bar is applied at about —20° to warm skin, the subdermal temperature falls to 0° in about 30 sec, and falls to about —6° by the end of 2 min. Thus, the superficial layers of the skin fall well below freezing point almost at once and super-cooling of the whole thickness of skin happens from about 30 sec onwards. This statement of the gradient of temperature will suffice to give a sufficiently clear idea of the amount of cooling happening in the skin in the observations now to be recorded. For these I shall now give the surface temperature only, for that alone was invariably known, the temperatures stated are not those at which the metal is applied, but those at which the surface of the metal becomes stable after its application. They are the highest temperatures of the skin surface prevailing over the period of cooling, which is also stated.

Temp of —15°   The bar has been maintained at this temperature for periods up to 10 min in a number of subjects , most applications of this duration are accompanied by no freezing   This degree of cooling results in no subsequent whealing,* and within an hour or less all trace of reddening of the skin has gone

Temp of —21°   After preliminary observations this has been finally tested in a number of young subjects, the duration of cooling varying from 10 to 120 sec   In all these tests freezing occurred once only, an observation that is excluded from the table

Subject	Temp	Duration in sec	After-effects	
			Wheal	Local redness
F	—21 5° —21 5° —21 5°	10 40 120	v slight slight fair	} No redness 4 hrs later
R	—20° —21° —21°	10 40 120	slight fair full	} Little redness in 2 hrs Slight redness till next day
B	—21° —21° —21°	10 40 120	slight full full	} Slight redness 4 hrs later
W	—21° —21° —21°	10 40 120	slight fair full	} No redness 4 hrs later
M	—21° —21° —21°	10 40 120	none none slight	} No redness 4 hrs later
G	—21° —21° —21°	10 40 120	slight fair full	} No redness 4 hrs later

As will be seen the results are nearly uniform   If cooling lasts 10 sec only, then there is no whealing or there is slight whealing   If cooling lasts 2 min then full whealing is the rule   These wheals subside within

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* Whealing followed in a single example previously recorded   no further example has been seen

about an hour, leaving a little redness behind, but this rarely lasts for more than a few hours

Temp of —24° to —25° The skin surface was cooled to this temperature in 13 young subjects, the bar being in place in each instance for precisely 10 sec In every instance but one, full whealing was seen in the cooled skin within 3 to 5 min In the exceptional instance the wheal was distinct but smaller, and this subject gave full and equal wheals when the skin was cooled for 40 sec and for 120 sec The skin examined 4 hrs later showed no trace of wheal or redness, with one exception in which a faint redness was still present 18 hrs after cooling

In several other subjects cooling of this extent was maintained for 40 sec and for 120 sec The wheals were similar to those from 10 sec cooling, but whereas in the latter all redness disappeared within a few hours, after the 2 min cooling a little redness was visible next day, though not subsequently

Temp of —30° A bar at this temperature can rarely be maintained on the skin for 5 sec without freezing it A 5 second contact without freezing is enough to produce distinct or full whealing The redness which follows subsidence of a wheal so produced does not last

Freezing

It is important to distinguish between two forms of injury, namely, that of supercooling and that of freezing and between the reactions of the skin to these two There are similarities and there are important differences between these reactions

First it should be made clear that whealing follows not only supercooling but freezing also, and that it is the frost and not the simultaneous supercooling which is responsible in the latter instance It has been seen that whealing may follow supercooling to temperatures of —20 to —25°C, but that at the first temperature cooling must last for minutes to give the full effect Supercooling at —15° has not been found adequate even when prolonged Now freezing often happens at this and at higher temperatures, namely, —10° or —5°C, and unless freezing at these temperatures is of short duration, and therefore only very superficial, whealing will always follow Thus while supercooling at —15° for 10 min or even longer, does not give wheals, freezing at this temperature for 20 or 30 sec always does Whealing is a reaction common to these two as well as to many other types of cutaneous injury

We come to differences in the reactions It should be understood that the lower the temperature of freezing and the longer it lasts, the more certainly will the skin wheal subsequently Thus short freezes of 5 sec at —15°, or of 1 or 2 sec at —25°, rarely yield wheals But all these freezes, even though whealing does not occur, are followed by conspicuous local reddening and tenderness of the skin, which persists for days and gives

place to pigmentation and to desquamation, it is true that, when supercooling fully wheals the skin, a little local redness may be left for 24 or even 48 hours, but this is its maximal and unusual time limit, whereas the mark of a freeze often persists for several weeks Thus if a forearm has been used for numerous tests of supercooling and freezing to day, the areas of skin that have been frozen are the only ones remaining clearly recognisable during the following days There is a fundamental difference between the reaction to frost and to cooling, while both wheal the skin, freezing produces additional effects, particularly persistent evidence of damage

It is to be emphasised in this connection that the fullness of whealing does not forecast the persistence of redness, these two reactions are unrelated quantitatively, a freeze which fails to wheal is followed by long persisting redness, the full wheal of supercooling is not Moreover, although freezing will itself give whealing, yet if skin is supercooled and happens to freeze in the last second or two of the time allotted, that skin will wheal less than skin that has been equally cooled but has not frozen In using the bar at —25°, and attempting to supercool with it for 120 sec, freezing not infrequently interrupts the observation, it was in these that it first appeared that freezing tends to reduce the full whealing expected from supercooling of this degree and duration The matter was brought to a final test by supercooling two areas at —25° each for 2 min, and in one of these at once freezing the same area for 1 second by applying to it a second bar at —30° Subsequently the wheals on the two areas were compared In each of four subjects, the control skin, that had been supercooled and not subsequently frozen, presented much the larger wheal, moreover, these wheals were accompanied by much the more intense and widespread flare It was interesting to note, in instances where the bar used to freeze and that used to supercool made contacts failing precisely to correspond, that the rim of supercooled skin that had escaped subsequent freezing, alone gave full whealing The meaning of this phenomenon is unclear, but it has its present importance in bringing convincing evidence that the reactions to supercooling and to freezing do not differ from each other merely in the degrees to which they injure the tissue

Excised skin

Repeated additional tests have been made on pieces of excised human skin, taken from post-mortem subjects one or two days after death and sometimes kept for a day or two longer in an ice chest The strip of skin is pinned out on cork, all obvious surface moisture removed, and the surface exposed for 15 to 30 minutes in an airy room to allow any further surface moisture to evaporate The skin is not allowed to lose its natural suppleness A small thermal junction is introduced immediately below the dermis and the copper bar, withdrawn from acetone at —20° to —22° is applied over it, repeatedly if necessary, until the desired temperatures are reached There

is no difficulty in reducing the temperature of the subdermal junction to —6° or —7°, and on a number of occasions temperatures of —9° and —9 5° have been reached, without the skin freezing   Lower temperatures have not been reached without the occurrence of freezing, which, when it occurs, is at once recognised by an abrupt rise of temperature of the junction by 1° or 2°, and is obvious as soon as the bar is removed

Thus, supercooling of live and dead skin presents no difference, both can be cooled so that the surface is at about —21° and the depth at about —9° without freezing happening

Comments

In a paper published a year ago (2) I showed that immersion of the hand in water of a certain coldness soon causes appreciable swelling of the limb   Such a reaction, measured by the swelling, is detectable when exposure is at a temperature as high as 18°C (approx), it is increased in degree as the temperature of the bath is lowered down to the region of freezing point   The reaction was ascribed in my earlier paper to direct injury by cold of the tissues, and especially the skin, concerned , and it was regarded as belonging to a number of reactions providing similar evidence One reaction, however, I was unwilling to stress in this connection, namely, the very occasional triple response which had been seen by Love and myself as an acute reaction in supercooled skin , and I was unable to do so because the precise conditions under which this reaction occurs could not then be defined , it was not clear that this response might not be exceptional or indeed abnormal   That is no longer the case, for, as the results here recorded show, full whealing is the rule, and obvious whealing invariable, after the superficies of the skin has been supercooled to —24° for 10 sec   Whealing is also the rule when cooling at —20° is continued for 2 minutes

Thus, there is a series of these direct reactions to cold, with a progress in intensity as they are traced from 18° downwards and past zero to the lower points of supercooling   These reactions are all ascribed to tissue injury and are at present regarded as fundamentally alike, being responses of the blood vessels to H-substance released from tissues damaged by cold Thus we may conceive slight grades of tissue damage at the higher temperatures, gradually and almost steadily increasing in intensity as freezing point is approached, but showing an abrupt rise of intensity as the lower temperatures of supercooling are reached   We cannot be very precise in naming the actual temperatures involved, because the tissues when supercooled in small mass for short time periods are not cooled to a uniform temperature but present a gradient from the surface   We may say, however, that the abrupt rise in cutaneous damage occurs when the average temperature of the skin in its thickness, has sunk to a point near —15°C

There is but one other comment that I desire to make   It will be manifest from these observations on supercooling that acute reactions of

the skin comprising transient reddening and swelling, after quite brief exposures to very low atmospheric temperatures, are not necessarily to be ascribed to frostbite, there is the definite possibility that many may be due to supercooling rather than to freezing of the skin

SUMMARY AND CONCLUSIONS

1 A method is described by which the surface of the human skin can usually be supercooled to —20°C or —25°C for short periods of time (10 to 12° sec) without freezing the skin Dead skin can be supercooled as well as living

2 Such cooling, especially to the lower temperature, is regularly followed in the living skin by the acute inflammatory reaction termed in previous publications the triple response

3 The immediate reaction of the skin to adequate supercooling and to actual and suitable freezing is similar, namely, the triple response

4 But freezing, if it happens to occur directly after adequate supercooling, tends to check the full development of the wheal which would otherwise follow supercooling Although whealing may be said to be a more prominent after-effect of supercooling than of freezing, yet the delayed after-effects of freezing are much the more conspicuous , it is clear that the heavier damage of frost introduces reactions that are far less easily reversed or repaired

5 It is suggested that the reactions described form part of one series, representing injury of skin, and that this starts as high in the temperature scale as 18°C , increases slowly in intensity as zero is approached, to rise abruptly to great intensity in the lower levels of supercooling

6 The acute reactions to supercooling must occur from time to time in exposure of the skin to low atmospheric temperatures and should be distinguished from true frostbite

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OPHTHALMOPLEGIA IN GRAVES' DISEASE

By F F RUNDLE* and C W WILSON †

(*From the Westminster Hospital School of Medicine*)

OPHTHALMOPLEGIA is an interesting and important sign in Graves' disease
for it both raises the question of the relationship between the ocular and
thyroid components of the syndrome and provides a tool for its investigation
The expression " ophthalmic type of Graves' disease " is used here to describe
cases in which the ocular changes occur without goitre or hyperthyroidism
Evidence previously adduced (11) suggests that such patients do in fact
belong to the class, Graves' disease   This paper is based on data from
patients of the purely ophthalmic type and from a random sample of the
ordinary type of Graves' disease with hyperthyroidism   The characteristics
of the ophthalmoplegia are defined and shown to be similar in the two groups

Method

The vertometer, an instrument for measuring duction movements of
the eye, and the technique of its use have been described elsewhere (13)
Elevation, depression, adduction and abduction of the eye are measured
from a standard central position in which the subject looks directly forward,
Reid's base line being horizontal
    Standards of ophthalmoplegia   It is convenient to adopt some value for
each duction movement at, and below which clear limitation (ophthalmo-
plegia) is indicated   Table I gives the mean values for the various duction
movements in normal controls and the ranges taken to indicate different
degrees of ophthalmoplegia
    Patients of the ophthalmic type fall into two sub-groups, post-thyroidec-
tomy (8 cases) and spontaneous (24 cases)   In the former ocular changes
were absent or slight before operation and only became severe afterwards **
In 6, thyroid function had been restored to normal, in 2 mild hypothyroidism
was present

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* Aided by a grant from the Medical Research Council

† Physicist in the department aided by the British Empire Cancer Campaign

** In thyrotoxic patients with marked ocular changes, the latter have been occasionally
observed to worsen perceptibly after thyroidectomy but such patients have been excluded from
the ophthalmic group

TABLE I

Normal range of eye movements and standards of ophthalmoplegia

Duction*	Right eye				Left eye			
	U	D	I	O	U	D	I	O
Average normal range and standard deviation (13)	43 4° ± 3 0°	50 2° ± 4 1°	46 2° ± 3 5°	48 6° ± 5 3°	42 3° ± 2 9°	50 0° ± 3 7°	49 4° ± 4 1°	47 1° ± 4 3°
Slight ophthalmoplegia	34°-30°	38°-34°	36°-32°	34°-30°	34°-30°	38°-34°	37°-33°	34°-30°
Moderate ophthalmoplegia	20°-29½°	24°-33½°	22°-31½°	20°-29½°	20°-29½°	24°-33½°	23°-32½°	20°-29½°
Severe ophthalmoplegia in range less than	20°	24°	22°	20°	20°	24°	23°	20°

* The letters U, D, I and O in this and subsequent tables indicate upward, downward, inward and outward movements of the eyes

We are indebted to Dr S P Meadows for referring to us, from an ophthalmic hospital, all but 3 of the patients who developed ocular changes spontaneously * They presented three main types, with exophthalmos and ophthalmoplegia as the chief signs, with lid retraction** (more often than not unilateral), and with marked exophthalmos but no ophthalmoplegia Evidence of goitre and hyperthyroidism was conspicuously lacking Five patients had suggestive general symptoms, but in 4 no clear evidence of hyperthyroidism ever developed while in the fifth, though well marked hyperthyroidism eventually supervened, it postdated the onset of unilateral lid retraction by four years

In the thyrotoxic group our aim was to examine a random hospital sample Therefore all patients with definite thyrotoxicosis presenting themselves at a general hospital during the period of investigation were included in the series except for 3 with cardiac failure No distinction was made between those with primary (diffuse) and secondary (nodular) toxic goitre or between those with eye signs and those without

* Two patients with goitre and hyperthyroidism as well as marked ocular changes have been referred from the same source but are excluded from the present group The proportion of 32 purely ophthalmic to 2 ophthalmic plus thyrotoxic forms probably greatly overstates the actual ratio attending even an ophthalmic hospital since the presence of goitre and hyperthyroidism would lead to their transfer elsewhere at an earlier stage But it is clear that such a special hospital provides a relative abundance of cases which are distinctly uncommon in general medicine

** Lid retraction is used in the sense, retraction of the free border of the upper lid to or above the level of the limbus, Reid's base line being horizontal and the gaze directed forwards

The contrast between the incidence and severity of ophthalmoplegia in the two groups is shown in Table II

TABLE II

Ophthalmoplegia in ophthalmic and thyrotoxic forms

Clinical group	Patients included	Ophthalmoplegia			
		absent	slight	moderate	severe
32 Ophthalmic	Males 11 \}total, 32 Females 21 Mean age 43 5 ± 12 2 years	7*	3	7	15
57 Thyrotoxic	Males 6 \}total, 57 Females 51 Mean age 39·9 ± 11 3 years	34	18	3	2

* Includes 2 patients with lid retraction only and 5 with marked exophthalmos, with or without lid retraction, but without ophthalmoplegia

The ophthalmic group

The incidence and degree of paralysis of the various eye movements may be represented diagrammatically as follows —
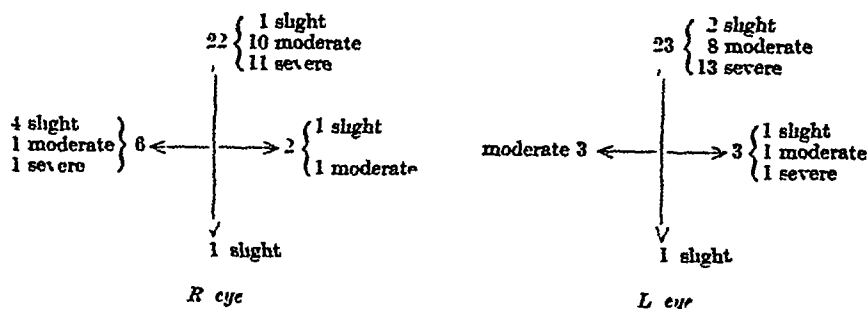
Symmetry of affection of the eyes   Paralysis of elevation tends to be bilateral and symmetrical   Thus in 21 of the 24 patients affected by this palsy it was bilateral   The degree of limitation also tends to be equal in the two eyes , this point is examined more fully below in connection with squint

Incidence of ophthalmoplegia on the various ductions   There is no suggestion that any particular movement is more likely to be paralysed in one eye than the other and we may therefore use the combined values for

the two eyes in comparing the incidence on the different ductions as
follows —

$$45 \begin{cases} 3 \text{ slight} \\ 18 \text{ moderate} \\ 24 \text{ severe} \end{cases}$$

$$\left. \begin{matrix} 5 \text{ slight} \\ 2 \text{ moderate} \\ 2 \text{ severe} \end{matrix} \right\} 9 \xleftarrow{\quad\text{out}\quad} \quad \text{in} \quad \xrightarrow{\hspace{2cm}} 5 \begin{cases} 1 \text{ slight} \\ 4 \text{ moderate} \end{cases}$$

2 slight

R and L eyes
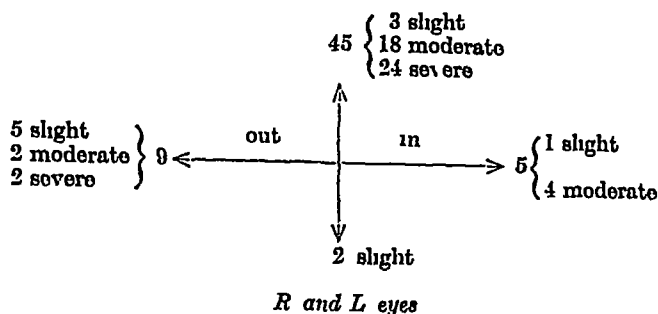
The frequency of paralysis of elevation is significantly greater than that of other movements * Considered separately the values for adduction, abduction and depression show no clear difference but, if the horizontal movements are taken together, the proportion of 14 to 2 shows that paralysis of one or other is more frequent than that of depression

The severity of paralysis has a similar distribution   Thus the proportion of severe paralyses is greater for elevation than for the other ductions taken together (24 in 45 as compared with 2 in 16)   In individuals also the severity of paralysis of elevation tends to overshadow that of other ductions   Thus in 9 eyes limitation of elevation was combined with that of other ductions, in 5 the degree of the former was greater than that of the latter and in the remaining 4, they were equal   In 20 eyes there was severe limitation of elevation without other paralysis, the converse obtained in one eye only where slight limitation of abduction was present without limitation of elevation **

The same pattern of paralysis has been observed in individual patients during the ingravescence of ocular changes (in several cases, elevation has become measurably less and even severely limited while all the other ductions remained full)   The most striking example occurred in a woman who rapidly developed exophthalmos and ophthalmoplegia after thyroidectomy for severe thyrotoxicosis   The data for her case are shown in Fig 1, smooth curves being drawn through successive measurements of the duction movements

* The significance of the frequencies in this and subsequent cases was examined by means of fourfold tables, Yates' adjustment being made (3)   Frequencies were regarded as significantly different if the value of χ^2 was greater than 3 841 (for 1 degree of freedom) namely, if the probability of their being similar was less than 5 per cent

** It is suggestive that in the only eye of the present series in which a horizontal duction (outwards) was completely paralysed, the eye squinted 32° downwards and could not be raised from that position more than 5°   Thus paralysis of elevation still far exceeded that of the horizontal duction in extent
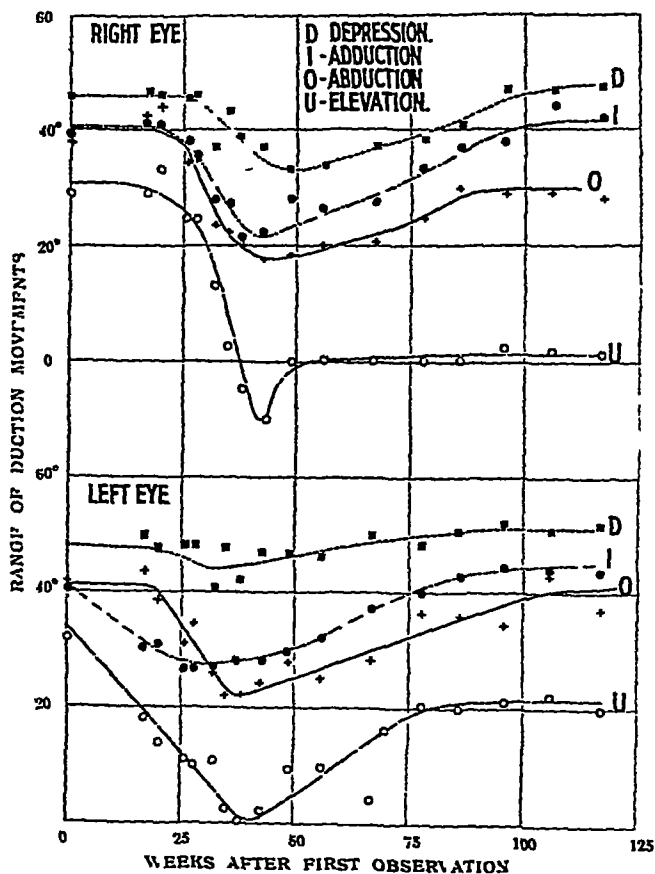
Fig I   Development and remission of ophthalmop'egia in a patient with severe ocular changes occurring after thyroidectomy   The first set of readings was taken a few days before the operation

Elevation of both eyes became completely paralysed, in fact, for a time the right eye could not even be raised to the central position and permanent complete paralysis of that duction supervened   Only 20° of elevation was regained in the left eye   Paralysis of horizontal ductions was never more than moderate and recovery was complete except for abduction on the right   Depression was only slightly affected and returned fully to normal   Naffziger (7) describes the same march of ophthalmoplegia in his severe post-operative cases

Pattern of squint   The term squint is used here to signify the presence of obvious asymmetry of the optic axes in the central position of the head

and gaze   It was present in 12 patients and was paralytic in type   The distribution of squints may be shown diagrammatically —
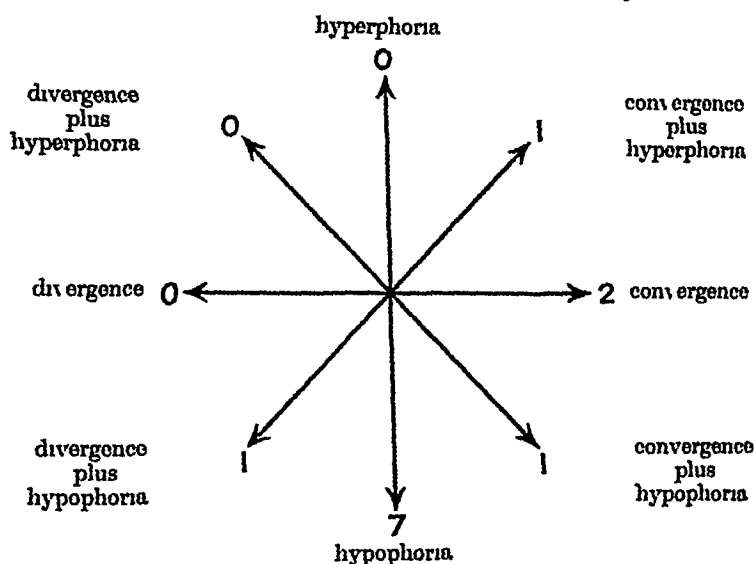
Hypophoria and convergence are commonest, corresponding to the greatest incidence of paralysis of elevation and abduction , but relative to the frequency of paralysis, horizontal squint is commoner than vertical

Asymmetry of muscle power rather than absolute paralysis appears to be the essential factor in the genesis of squint   Thus the mean difference in elevation of the two eyes in the 7 patients with hypophoria was 21 4° ± 13 7° compared with that of 2 8° ± 3 8° in those without vertical squint But there is no absolute loss of elevation for with the " master eye " screened, the hypophoric eye usually " fixed " immediately and often a further range of elevation was possible though it was less full than in the master eye   By contrast, there is no squint, even with severe bilateral paralysis of elevation, provided limitation remains symmetrical   On the other hand paralysis of the horizontal ductions, even when slight, usually results in squint because it does not tend to affect the dextro- and lævorotators symmetrically

The paralytic character of the squint and the importance of asymmetry of muscular power are strikingly demonstrated in the patient whose data are shown in Fig 1   When first seen muscle balance was normal and there was no squint, though the left eye was highly myopic, which probably facilitated the subsequent paralytic dissociations   The vertical and horizontal components may be described separately   Severe paralysis of elevation of the left eye antedated that in the right by several months and she first developed downward squint on the left   Later when elevation became equally limited on the two sides, squint was lost   Finally with unequal recovery of elevation it recurred, the left eye passing into hyperphoria   Similarly early paralysis of adduction (left) caused that eye

to diverge but later with the more or less equal involvement of all the horizontal ductions this squint was lost   Finally the left eye passed into well marked convergence because of persistent paralysis of abduction on the right   The effort of using the more paralysed right eye is uneconomical and unusual but presumably depends on myopia and poor visual acuity on the left

Torticollis and lid retraction   Squint is a disabling complication and compensatory torticollis develops unless the squinting eye is shielded Characteristically the neck is hyperextended so that though the patient looks forwards the visual axes are depressed relative to Reid's base line , conjugate vision is thus maintained   But torticollis is also common in severe bilateral paralysis of elevation without squint   Though with an effort the eyes may be raised to, or even beyond, the horizontal the patient is only comfortable with the visual axes 15° to 20° below the central position and torticollis in extension develops

Habitual depression of the gaze and torticollis have, in turn, an important bearing on the behaviour of the upper lid   Lid retraction due to spasm of the levator palpebræ causes a widening band of sclera to be exposed between the free margin of the upper lid and limbus as the eye turns downwards (8) When such lid retraction is combined with torticollis and habitual depression of the gaze, the impression of an exaggerated degree of lid retraction and exophthalmos is created, but the characteristic posture of the head will indicate the presence of paralysis of elevation

Another type of lid retraction present in the central position and due to over-action of the levator palpebræ in the presence of superior rectus weakness passes off when no effort is made to hold the eyes up, as in the position of rest   The lid may even appear ptosed   But though often stated to occur with the ophthalmoplegias of Graves' disease, ptosis of the upper lid is not found if the patient's head is first placed in the correct anatomical position *

Functional deficiencies in the extrinsic muscles   Though at least five muscles co-operate in both adduction and abduction of the eye, the internal and external recti have by far the greatest effect and we may assume that paralysis of the horizontal ductions in Graves' disease is due to deficiency of these muscles

With limited elevation, however, the case is less simple and since this is the principal ophthalmoplegia it may be examined in more detail   Extreme elevation is regularly associated in these patients with a distinct sense of strain and muscular fatigue, and may excite conspicuous blinking and watering of the eyes   One patient worked in a tobacconist's shop and generally sat down behind the counter   But she noticed her eyes becoming easily tired and soon felt the strain if she had to look up for long, as when a

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* The illusion of ptosis is presumably created because the fissure normally narrows in the down turned position of the eyes   In some cases also protrusion of the upper lid by fat causes its free border to lie at an unusually low level relative to the cornea when the levator is not contracting

customer stayed to talk She found she was always looking down as this was the only way in which she could get relief from the feeling of strain

Elevation of the eye depends upon the combined action of two muscles, the superior rectus and inferior oblique Paralysis of the former is primarily and often solely the cause of the deficient elevation Instances of superior rectus paralysis in Graves' disease have been reported previously (5, 6, 10, 14, 15), but the peculiar frequency and importance of this palsy have not been sufficiently emphasized

The signs of superior rectus paralysis depend on the following anatomical data — the long axis of the superior rectus makes an angle of about 25° with the sagittal plane of the globe (16) and hence this muscle is only fully efficient as an elevator in moderate abduction The axis of the inferior oblique cuts that of the globe at an angle of nearly 40° and it is only fully efficient as an elevator in extreme adduction The relative contributions to elevation made by the two muscles therefore depend on the position of the globe, As the upturned eye passes from the fully adducted to the moderately abducted position the burden of elevating it passes smoothly and completely from the inferior oblique to the superior rectus In abduction the former acts simply as an abductor The eye is kept straight on its horizontal course solely by the counteracting pulls of the superior and inferior recti Hence if the former is paralysed the eye tends to " drop " sharply in full abduction In movements straight up from the central position the power of the superior rectus predominates (2) Isolated superior rectus palsy may therefore be distinguished as follows — in full adduction elevation is normal (if the other eye is healthy the axes of the two will be conjugate in this position), from the central position, severely limited and in moderate abduction, absent , in full abduction the visual axis tends to drop sharply * Maximum asymmetry is present in efforts to look upwards and outwards towards the affected side When inferior oblique paralysis is superadded, elevation from the central position approaches zero and the eye tends to " drop " also in full adduction

It is possible by carefully examining the range of elevation from the central position, in full adduction and in moderate abduction, and the plane of the eye in full abduction, to estimate roughly the relative importance of the superior rectus and inferior oblique in the ophthalmoplegia of elevation In 9 eyes paralysis, partial or complete, of the superior rectus occurred alone , in no case was there isolated paralysis of the inferior oblique When both muscles were involved elevation upwards and inwards was nearly

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* The apex of the cornea may drop 25° or more and close observation will usually show this to be associated with distinct intorsion of the eye Looking fully round to the affected side may thus cause intense diplopia and vertigo One such patient said that he felt unsafe out of doors unless the affected right eye was screened His compensatory torticollis indoors was noteworthy The neck was hyperextended and turned slightly to the left, his body was turned well round to the right so that when looking in the desired direction his eyes were in moderate laevoversion and the paralysed right eye in adduction In this way conjugacy was maintained and diplopia avoided

always freer than upwards and outwards, indicating that weakness of the inferior oblique was relatively less than that of the superior rectus   Again in 3 eyes in which ophthalmoplegia, at first slight, was observed to progress steadily and become complete, elevation in moderate abduction was lost before that in full adduction   Thus we may conclude that superior rectus paralysis is the dominant factor in deficient elevation and that it is highly characteristic of ophthalmoplegia in Graves' disease *

The thyrotoxic group

Incidence of ophthalmoplegia   23 of the 57 patients had limitation of one or more ductions   Ophthalmoplegia was generally mild, moderate or severe degrees occurring in only 5 cases   Mild or moderate degrees of ophthalmoplegia frequently occurred in the absence of lid retraction (13 eyes), exophthalmos (3 eyes) or both (6 eyes) †

The incidence may again be represented diagrammatically —

R eye	L eye	R and L eyes
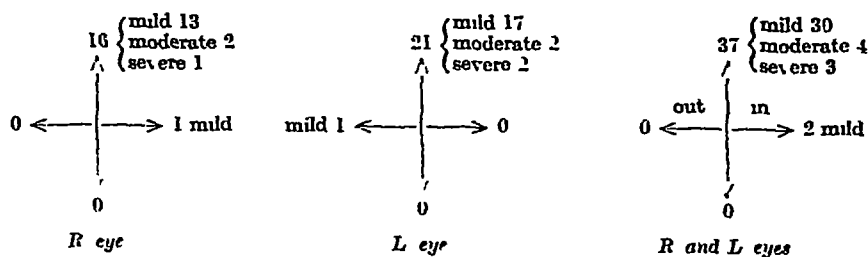
As in the ophthalmic group, paralysis of elevation is clearly more frequent than that of other ductions   Again it tends to be bilateral and symmetrical , it was bilateral in two-thirds of the cases, a proportion higher than is likely to occur by chance, and the mean difference for elevation in those with paralysis was only $3\ 8° \pm 5\ 6°$, a value not significantly greater than the corresponding figure in controls

Thus limitation of elevation of the eyes is not uncommon in thyrotoxicosis   But it is slight and generally overlooked because of its symmetry and the consequent absence of diplopia ‡

The data in Tables III and IV both confirm the predominant involvement of elevation and suggest that, as in the ophthalmic group,

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* Because of the tendency to unequal involvement of the superior rectus and inferior oblique it is important in measuring elevation to ensure that the eve is raised straight up from the central position   Patients who retain power in the inferior oblique but have lost it in the superior rectus may, in forced elevation adopt a   trick movement   adducting as well as elevating the eye

† An exophthalmometer reading below the normal average was taken as indicating the absence of exophthalmos

‡ Deficient elevation may pass unnoticed on clinical examination if compensatory torticollis in extension and relative depression of the gaze are uncorrected   Elevation will then be tested from below the true central position and its range overestimated

horizontal ductions tend to be more affected than depression The mean values for adduction and abduction (Table III) are significantly decreased, that for depression may be increased slightly * Table IV gives the frequencies of limitation short of ophthalmoplegia, the range examined being that between 2 and 3 times the standard deviation below the normal average The combined frequencies of adduction and abduction probably exceed that of depression

The two patients from the thyrotoxic group with most severe paralysis also provide striking evidence Their eye movements are shown in Table V

TABLE III

Range of eye movements in thyrotoxicosis and differences from normal

Duction	Right eye				Left eye			
	U	D	I	O	U	D	I	O
Average range and standard deviation in thyrotoxicosis	36 8° ±6 5°	52 0° ±5 2°	44 4° ±4 1°	46 3° ±4 4°	35 5° ±6 1°	52 5° ±5 3°	46 6° ±4 2°	45 3° ±3 3°
Average normal range minus average in thyrotoxicosis *	+6 6°	—1 8°	+1 8°	+3 3°	+6 8°	—2 5°	+2 8°	+1 8°

* Probability that the difference is not significant is less than 2 per cent in each case

In Case 1, the left eye squinted downwards and tests of elevation in different planes showed a preponderant affection of the superior rectus In Case 2, the right eye squinted downwards and outwards , on this side both superior rectus and inferior oblique were considerably paralysed but on the left weakness of the superior rectus was clearly greater In Case 2 paralysis of horizontal ductions was present but slight compared with that of elevation Thus the characters of the ophthalmoplegia are identical with those found in well-marked examples within the ophthalmic group But in both cases goitre and well-defined hyperthyroidism were present Indeed, in Case 1, hyperthyroidism was severe She had lost 20 lbs in weight in spite of an enormous appetite , tremor, emotionalism, tachycardia, heat intolerance and fatiguability were all pronounced and she had finally been

* The mean increase in depression is very small and though it may be due to overaction of the depressors in the presence of weak elevators, a systematic variation resulting from a minor alteration in technique cannot be excluded

TABLE IV

Probability of slight limitation of eye movements in control and thyrotoxic subjects

Duction	U	D	I	O
No of control eyes within range (200 eyes)	4	1	0	0
No of thyrotoxic eyes within range (113 eyes)	28	4	8	6
χ^2 value* (1 degree of freedom)	40 3	3 99	13 8	10 0

* See previous footnote

TABLE V

Severe ophthalmoplegia in thyrotoxicosis

	Eye movements							
	Right eye				Left eye			
	U	D	I	O	U	D	I	O
Case 1	$34\frac{1}{4}°$	59°	41°	46°	$12\frac{1}{4}°$	61°	49°	43°
Case 2	0°	48°	31°	39°	$18\frac{1}{2}°$	52°	35°	$47\frac{1}{2}°$

compelled to give up her work   In both, thyroidectomy produced great amelioration of the general condition

    Correlation of elevation to age   An examination of the data relating to the movement chiefly affected, namely elevation, has shown this movement to decrease significantly with increasing age *

Discussion

    Correlation between clinical and pathological findings   The extrinsic muscles regularly show a greatly increased fat content in thyrotoxicosis (12) and it has been here established by measurement that functional deficiency is also relatively common   Moreover, like the increased muscle fat,

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* The correlation coefficient was — 0 358 ± 0 083

ophthalmoplegia may occur in the absence of exophthalmos and lid retraction The range of elevation of the eye is also correlated to age as is the fat content of normal eye muscles The fat content in thyrotoxicosis is proportional to the normal fat content of the muscle and is highest in the levator palpebræ, spasm of which causes lid retraction, one of the commonest clinical signs But there the particular correspondence ends for the fat contents of the different recti are not correlated with their relative tendency to paralysis

Relationship of ophthalmoplegia and hyperthyroidism It is clear that in the two groups examined, ophthalmoplegia is similar in kind but differs in degree In the one, a random hospital sample of thyrotoxicosis, it is mild In the other, the ophthalmic group, it is more frequent and severe while thyroid function is normal Thus a general inverse relationship is demonstrable between the ophthalmoplegia and thyroid manifestations But there is much overlapping of the two components in individual cases and clearly all the patients are most simply regarded as belonging to the same disease class In particular there is ample evidence, both clinical and pathological, that disorders in the extrinsic muscles are an integral feature even of patients at the thyrotoxic end of the range Thus no support has been found for Brain's claim (1) that " exophthalmic ophthalmoplegia " is a syndrome separate and distinct from Graves' disease More recently Hertz, Means and Williams (4) have developed the thesis that there are two different types of Graves' disease, the classical form and an " ophthalmopathic form " In the former there is exophthalmos and lid lag, in the latter, ophthalmoplegia and severe œdema of the orbital and other tissues But their theory is largely based on a false premiss, namely that œdema of the orbital tissues is the proximate cause of exophthalmos

As suggested previously (11) the type characterized by ocular changes without hyperthyroidism is best regarded clinically as a variant of Graves' disease and not as a syndrome distinct from it This unifying concept is strongly supported by the present data but it raises an awkward problem in nomenclature If we classify all our patients as suffering from Graves' disease, referring to ophthalmic and thyrotoxic forms, we employ the expression in a distinctly wider sense than custom sanctions Thus many ophthalmic forms have no trace of hyperthyroidism whatever and the term Graves' disease has always implied this Nor is it legitimate to include the ophthalmic forms under some sub-title such as " exophthalmic ophthalmoplegia " (1) or " malignant exophthalmos " (9) since, as stated, both ophthalmoplegia and exophthalmos may be absent

Conclusions

1 Ophthalmoplegia in Graves' disease tends to be bilateral and has a well-defined pattern Paralysis of elevation is of outstanding frequency

and severity It depends primarily on weakness of the superior rectus muscle Taken together the horizontal ductions are more liable to paralysis than depression Paralytic squint is a frequent complication and depends on asymmetry in the power of corresponding recti

2 There is a general inverse relationship between ophthalmoplegia and hyperthyroidism in Graves' disease The expression, "Graves' disease," may conveniently be used to include ophthalmic forms, in which hyperthyroidism is slight or absent, as well as ordinary thyrotoxicosis Disorders of the eye muscles are an integral feature of both In the ophthalmic forms ophthalmoplegia differs in degree not in kind from that in thyrotoxicosis

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BULGING OF THE EYELIDS WITH EXOPHTHALMOS

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REFERENCE has been made (4) to fatty swelling of the lids occurring with exophthalmos in thyrotoxicosis In this paper its clinical characteristics and mechanism are more fully examin.d Such swelling is not confined to patients with Graves' disease but is found in a variety of conditions characterized by overfilling of the orbit and exophthalmos It is, in fact, simply a protrusion of the normal lid and is not due to œdema of the palpebral tissues as generally stated The term, " exophthalmos," is used here to denote abnormal protrusion of the eyeball

Normally the cone-shaped, bony cavity of the orbit is filled by the globe and its adnexa 'Only the anterior boundary, formed by the globe and lids, is mobile and distensible without destruction Variations in its plane, in the direction of protrusion or recession, accurately reflect changes in the degree of orbital filling Even the smallest increases necessarily cause some protrusion of the globe and lids Exophthalmos is an arresting phenomenon but the cognate sign, lid protrusion, to which far less attention has been paid, is at least equally important The latter has well-defined clinical appearances and is not subject to the vagaries that influence the appearance of exophthalmos It thus provides a solid basis for estimating the extent of pathological conditions in the orbit

Variations in the plane of the lids

Normal relationship of the globe and lids The plane of the orbital opening or outlet is taken as the vertical plane passing through the medial and lateral orbital margins at their mid-points It cuts the eyeball approximately at its equator where the diameter averages 23 mm The shape and area of the opening are roughly those of a circle of diameter 38 mm (5) Thus the eye occupies only some four-tenths of the opening, the major part of its extent is taken up by extra-ocular tissues lying directly deep to the lids

In normal subjects, when the eye is lightly closed, the globe forms a rounded, centrally-placed elevation Below and on either side the lids pass smoothly off it and he in the plane of the cheek and commissures where they cover the peribulbar tissues Above there is a groove between the prominence of the globe and the supra-orbital ridge Its depth varies but it is usually shallow

Recession of the lids In wasting of the orbital tissues the eye is sunken * The lids are receded where they lie on the adnexa round the globe Instead of a shallow groove above, there is a deep crevasse extending up under the orbital margin (Fig 5) This crevasse becomes rapidly shallower towards the commissures, a distinct localized recess may be seen in the region below the level of the trochlea on the inner side The lower lid is also sunken to a deeper plane than the skin overlying the inferior orbital margin especially towards the outer side

Bulging of the lids Bulging of the lids due to prolapsing fat has well-defined clinical appearances In the upper lid these are best observed with the eye closed, in the lower with the eye open Slight, moderate and considerable degrees of bulging are roughly distinguishable

Slight bulging of the upper lid is generally first apparent above and lateral to the prominence of the globe, the normal supra-bulbar groove being lost A localized dome-shaped swelling also appears in the infra-trochlear region of the lid † In moderate degrees of bulging the latter enlarges and extends laterally to unite with the fullness above the globe The junction between the two may result in a narrow sausage shaped swelling of the lid above and to the inner side of the prominence due to the globe When swelling of the lid is considerable the infra-trochlear bulge is generally very marked and extends down to the lid margin where it may become pendulous and overhang the skin of the medial commissure, hiding this and the inner canthus as in the striking examples illustrated byBurch(1) and Duthie (2) Passing laterally the bulge has a full, rounded contour and is widely continuous with that in the supra-bulbar region In profile the latter appears as a fold raised distinctly beyond the level of the eyebrow and globe Seen from in front it appears broad and sausage-shaped, lolling transversely down over the tarsal part of the lid from which it is separated by a crease of variable depth and distinctness (Fig 7) With the eye open, it may even overlap the lid margin and eyelashes ‡ Occasionally

the whole lid appears uniformly and grossly distended and its surface extent much greater than normal (Fig 10)   In profile its outline then sweeps down in a full unbroken arc incorporating the prominence due to the globe Lateral to the supra-bulbar region, the fullness broadens and extends on to the forehead in the region of the lateral angular process

In the lower lid slight bulging is indicated when its surface is raised above that of the adjacent cheek   Generally there is a firm, localized swelling just above the junction of the inferior and lateral orbital margins In moderate degrees the bulging extends medio-laterally and widens from above down   When considerable, it involves the whole of the lid but is usually most marked laterally where it becomes pendulous and overlaps the malar bone *   Occasionally two bulges are distinguishable in the general fullness of the lower lid, the one medially the other laterally

Prolapsing orbital fat as the cause of the bulging

It is quite clear that the bulging is due not to œdema of the palpebral tissues but to protrusion by orbital fat   Evidence for this has been adduced elsewhere (4) and may be supplemented here   In marked degrees of bulging of the lower lid the prolapsed orbital tissue can be felt, as the finger-tips press lightly downwards and backwards, slipping back over the inferior orbital margin, into the orbit   Concomitantly the globe protrudes by as much as 3 mm   Occasionally too in the upper lid the margin of the tissue prolapsing on to the lateral angular process can be felt as it is reduced into the orbit

The behaviour of these swellings with duction movements of the eye is also highly significant and is best observed in the lower lid   Here, elevation is normally associated with slight bulging, depression with recession and creasing, these changes being clearly due to corresponding forward and backward rotation of the fatty adnexa below the globe , this fact can be verified by simply retracting the lid and observing the movements of the adnexa during elevation and depression   When the lid is already bulged, the bulging is accentuated by elevation, diminished by depression (Fig 11 and 12)   The changes reproduce in exaggerated form those occurring in the normal lid with these ductions, being such as would result from *excess of fatty adnexa* below the globe   Moreover the agent responsible for the bulging is clearly deep to the lid and not in its substance

Finally, careful post-mortem dissections were made in two cases of Graves' disease in which considerable bulging was present   The findings

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* It is clear from dissections that the orbital fat is more free to overlap the bones adjacent to the orbital margin laterally than medially both above and below   On the inner side below the dense fascia binding the skin of the cheek to the malar bone extends right up to the orbital margin before giving place to the lax orbital septum but on the outer side it stops short some distance from it   Above and laterally also the septum appears to become stretched forming a recess between the skin of the eyebrow region and the lateral angular process

were similar in both The upper and lower lids were found to be normal in thickness and no evidence of excess fluid was found in the planes of dissection Deep to the upper lid lay a large, smooth comma-shaped mass of fat Its globose head occupied the infra-trochlear region and its broad tail extended laterally above the levator expansion * When the lid fell back into place it was evident that this fatty mass was the proximate cause of its bulging Similarly, deep to the lower lid an abundant fatty mass protruded and overhung the malar bone Further dissection, after removing the roof of the orbit, showed that this subpalpebral fat was directly continuous with, and merely formed a forward extension of, the peri- and retrobulbar fat of the orbit proper

Extension forwards occurs through three principal anatomical hiatuses (i) between the superior orbital margin and levator palpebræ expansion (ii) around the terminal ophthalmic vessels below the trochlea and tendon of the superior oblique and (iii) between the inferior oblique muscle, ligament of Lockwood and lateral palpebral ligament above and the infero-lateral margin of the orbit below There is a subsidiary hiatus (iv) below, which lies above and medial to the inferior oblique (Fig 1)
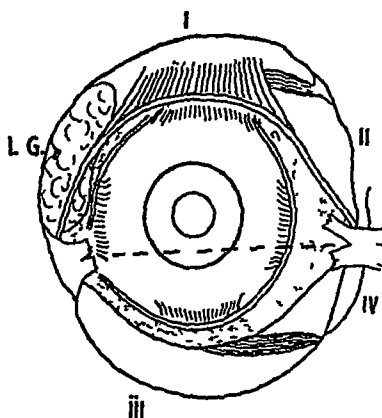
Fig 1   Diagram of deep relations of the lids The interrupted line indicates the level of the closed palpebral fissure Fascial adnexa of the globe are stippled, extrinsic muscles hatched, and the peripheral zone, where the lids lie directly on orbital fat, is dead white The numbers (i) - (iv) indicate the corresponding hiatuses The lacrimal gland (L G) may also become obscured by fat when the lids are bulged The levator palpebræ expansion is seen in cut section

All these hiatuses lead into " spaces " bounded anteriorly by the deep fascia of the lids Those corresponding to the first and second hiatuses are continuous round the medial border of the levator expansion Similarly when the lower lid is displaced forwards the two deep to it become confluent in front of the inferior oblique muscle

* In one case, the infra trochlear mass was distinctly dome shaped and not directly continuous with that above the levator

Differentiation from oedema of the lids Effusions readily occur into the lax layer between the skin and orbicularis oculi and the resulting swelling takes roughly the same shape as in marked protrusion by fat But pitting on pressure is readily elicited especially in the lower lid which can be compressed against the malar bone In the upper lid, gentle pressure against the globe, with the eye closed, also results in a well-defined imprint if much oedema is present However marked the bulging in Graves' disease, the lids do not pit on pressure

Oedema affects the lids diffusely, extends to their free borders and may also involve the neighbouring cheek or temple though here, the subcutaneous tissue being denser, swelling is less pronounced Duction movements have very little effect on marked oedematous swelling presumably because the oedema fluid is subcuticular and superficial to the orbital septum which, in the lower lid, is drawn in on depression

Gravity strongly influences the distribution of swelling due to oedema but that of fatty protrusion is governed by anatomical factors Thus it may be distinctly localized and even when generalized it is lumpy and most evident towards the orbital margins (Fig 6) ‡

TABLE I

Correlation between exophthalmometer reading and degree of lid protrusion (173 eyes)
The figures give the total number of eyes with the reading and protrusion indicated
One of the 87 patients had one eye only

Lid protrusion.* Exophthalmometer reading in mm †	—2	—1	0	+1	+2	+3	+4	+5	+6	Mean lid protrusion
12 0—13 75	2	2	2							—1
14 0—15 75	2	2	15							—0 3
16 0—17 75		2	21	6	1					+0 2
18 0—19 75			10	9	3	1				+0-8
20 0—21 75			4	14	9	7				+1 6
22 0—23 75			2	8	8	3	4	3	1	+2 4
24 0—25 75					1	6	1	3	3	+4 1
26 0—27 75					1	8	3	3	3	+4 0

* Values —2 to +6 represent the total degree of lid protrusion and were derived as follows — if the lid were sunken to any degree it was given a weight of —1 if neither sunken nor full, a weight of 0 if protruded slightly moderately or considerable weights of +1 —2 +3 respectively The total value for any eye may thus range between —2 and +6

† The exophthalmometer readings were made with Hertel s instrument from the lateral orbital margins the average value in controls by the same method is 16 9 — 1 8 mm The upper normal limit is not less than 23 0 mm

‡ In a large number of patients with Graves disease bulging of the lids has been found, without exception to be due to protrusion by peribulbar fat In a small but random sample of 6 patients with orbital tumour it was clearly due to protrusion by fat in 5 In the sixth there was severe oedema the growth had infiltrated the subpalpebral tissues on the inner side and ulcerated at one point near the medial commissure

Correlation between degree of lid protrusion and proptosis

It was noted previously that when the lids are bulged, pressure back on any one of the three structures, globe, upper or lower lid causes corresponding, further protrusion of the other two (4)   The globe and lids may evidently be regarded as interdependent parts of a highly mobile anterior " diaphragm " with respect to the orbital tissues

The correlation between lid protrusion and exophthalmometer reading, in a random sample of 87 patients with Graves' disease is shown in Table I * Low readings are associated with normal or sunken lids and high readings with protruded lids   But the correlation is shown even more convincingly in individual patients who provide their own control, namely those with unilateral exophthalmos and those observed during the development and remission of exophthalmos   In the former the unaffected eye provides the base line and a correlative degree of lid protrusion is found associated with the proptosed eye   In the latter, protrusion of the lids is seen to develop concomitantly with exophthalmos   Thus in one patient with Graves' disease, there was only slight bulging of the left upper lid and no clear exophthalmos at her first attendance   Subsequently she developed 6-7 mm of exophthalmos and considerable bulging of all four lids   Partial recession of the eyes was then accompanied by diminution of the bulging   In a similar case moderate fullness of the upper lids and exophthalmos were already present when she first attended   With the further development of 4 mm of exophthalmos, protrusion of all four lids became considerable (Fig 9 and 10)

Finally in a case of venous tumour of the orbit compression of the cervical veins regularly caused severe exophthalmos   The lids could be observed protruding *pari passu* with the globe and both were considerably bulged when exophthalmos became maximal   When pressure on the veins was released the globe and lids receded together and both finally became sunken

Mechanics of exophthalmos and lid protrusion

It has been shown (4) that the position of the globe is determined by the degree of filling of the orbit and clearly this may also determine the plane of the lids   The mechanism of both exophthalmos and lid protrusion can be examined experimentally by injecting molten paraffin wax (melting point 40°C) into the retrobulbar space in the dead body   The wax is injected with a record syringe and wide-bore, aspirating needle, 1 c c at a time   The needle is introduced through the lateral palpebral raphe and pushed quickly back towards the apex of the orbit before driving the plunger home   Speed

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* The same correlation is found in controls, it must be emphasized that the range of prominence of the eyeball and lids in control subjects is very wide and overlaps that in patients with exophthalmos   Thus slight or moderate bulging of the lids is not uncommon in controls especially if obese, the exophthalmometer reading is then above the normal average, as is, presumably too, the degree of orbital filling   But the considerable degrees of lid protrusion described here did not occur in any of a group of 52 controls taken at random

is essential, otherwise wax solidifies in the needle   Between injections, wax, syringe and needle are kept warm over a spirit-lamp

Visible sharp protrusion of the globe and lids occurs during the actual injections

Protrusion of the globe   The proptosis occurring in a series of experiments is shown in Fig 2 in which the exophthalmometer readings are plotted against the volume of solid wax injected
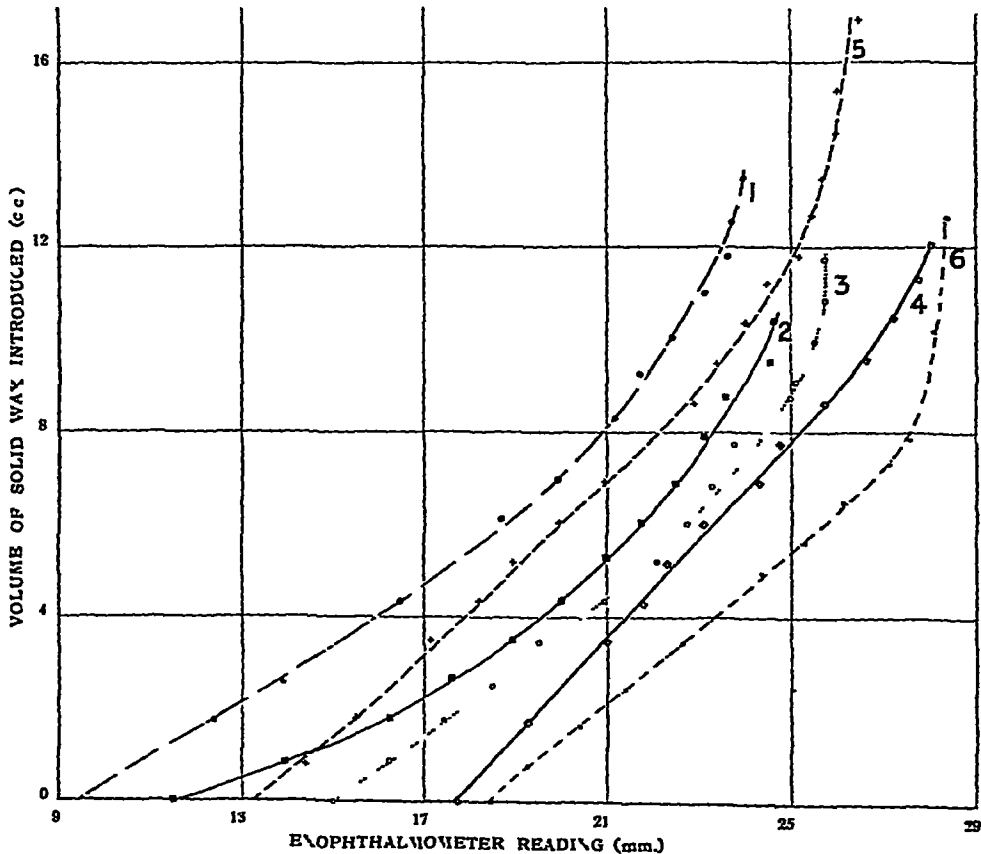
Fig 2   Proptosis resulting from the injection of wax into the retrobulbar space post mortem Each curve represents one experiment, ocular protrusion (mm) being plotted against the volume (c c) of solid wax introduced.

The general shape of the curves is similar , the greatest protrusion per unit of wax injected occurs in the initial stage   There is then a transition, more or less gradual, to a terminal phase in which the protrusion per injected unit is much reduced

Protrusion of the lids The changing appearances of the lids as injections proceed are strikingly similar to those seen in cases of Graves' disease and of orbital tumour Thus where the lids are originally sunken they first become normal, then pass through all degrees of bulging, in each experiment its degree eventually exceeds any seen clinically Terminally, in fact, it is mainly the lids which protrude

Table II shows the degree of lid protrusion and proptosis associated with different ranges of orbital filling in six experiments

TABLE II

Correlation between Hertel exophthalmometer reading, orbital filling and protrusion of lids

Mean Hertel reading mm	9 9*	16 1	19 6	23 0	26 2	27 2
Orbital filling (per cent)	51–60	61–70	71–80	81–90	91–100	101–110
Mean degree of lid protrusion †	—2 0	—0 4	+1 7	+3 8	+5 6	> +6 0

* Based on curve 1 (Fig 2) only

† The value, > +6, means that both lids were considerably swollen, by clinical standards, at a filling index lower than this

Thus, when the lids were receded the orbital contents occupied less than 60 per cent of the cavity, their anterior plane was normal at about 70 per cent filling and considerably bulged at more than 95 per cent filling *

Associated changes deserve mention In the later stages of each experiment, the upper lid became distinctly ptosed and covered most of the cornea Close observation during the actual injections showed it to drop sharply while the lower lid retracted slightly

When overfilling of the orbit became extreme the lacrimal caruncle and conjunctiva of the medial, inferior and lateral fornices, but not the superior, appeared to be crowded forwards The caruncle protruded through the fissure and the conjunctiva became thrown up into a fold along the free border of the lower lid Even post-mortem this heaped-up redundant conjunctiva gave the strong visual impression of œdema

Within the limits of a post-mortem experiment, the mechanical conditions produced in the orbit exactly correspond to those in solid orbital tumour It is noteworthy that after the injection of 5 to 10 c c of wax, the resistance of the globe to light digital pressure back became stony hard whereas previously it was soft and yielding Thus a classical sign of orbital

* The first two percentages correspond to the degrees of filling found in wasted and normal subjects respectively (4)

tumour was reproduced presumably because solidified wax already impinged on the globe   At the end of the experiments dissection showed the orbital
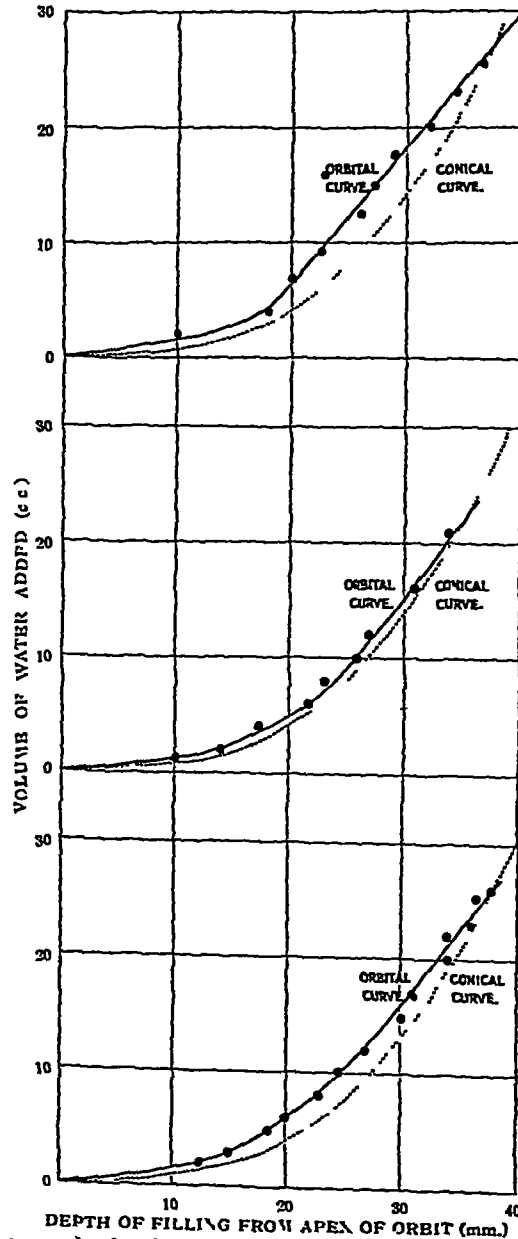
Fig 3   Curves showing depth volume relationships of three orbits and corresponding perfect cones   Each orbit was filled to the maximum extent possible   depth (mm.) and volume (c c) readings were taken during the process and are plotted.   The angle of the corresponding cone is determined from the final readings obtained in each case

cavity to be tightly packed with wax which largely disrupted and replaced the normal structures   It pressed on the posterior aspect of the globe and pushed the peribulbar fatty tissue forwards in a thin layer beneath the lids

Depth-volume relationship as the orbit fills   A depth-volume curve of the orbit (hereinafter referred to simply as an orbital curve) may be constructed as follows —the orbital contents are removed and leakage prevented by smearing the walls with molten wax   The orbit is then gradually filled with water from a record syringe, 2 c c being run in at a time   After each addition the distance of the surface of the water from the outlet is measured with a depth gauge , thus the depth of filling is derived

Orbital curves from a series of post-mortems may be compared with those of the corresponding perfect cones (Fig 3)   The angle of the cone is derived in each case from the final readings for depth and volume of the orbit   A theoretical curve (the conical curve) of the volumes corresponding to different depths can thus be constructed

Each orbital curve resembles that of the corresponding cone but close examination shows that its final part is practically a straight line   Thus beyond a certain point the volume/depth ratio is constant   Beyond that point the area of cross-section must therefore be the same   Thus the posterior part of the orbit fills as a cone, the fore part as a cylindroid *

Comparison of overfilling curve with other curves   This may first be made with respect to a particular orbit, data from which are plotted in Fig 4   The orbital and conical curves were obtained as for the orbits in Fig 3   Since volume is plotted against depth, the gradient of the curves clearly depends on the area of cross-section of the advancing base   Changes in gradient describe changes in area of the protruding section   The point, D, indicates the depth to which soft tissues filled the orbit before injections of wax commenced   The gradient of various curves beyond D may now be compared

Four different curves are shown, simple extrapolations of the orbital and conical curves (the extended orbital and extended conical curves), the curve actually obtained when wax was injected† (the overfilling curve) and the curve that would result if the injections caused protrusion of the eyeball alone (ocular curve) ‡   Both the ocular and extended orbital curves represent elongating cylinders , the sectional area of the former is

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that of the globe only, that of the latter is equal to the whole available area of the orbit at and immediately behind the plane D
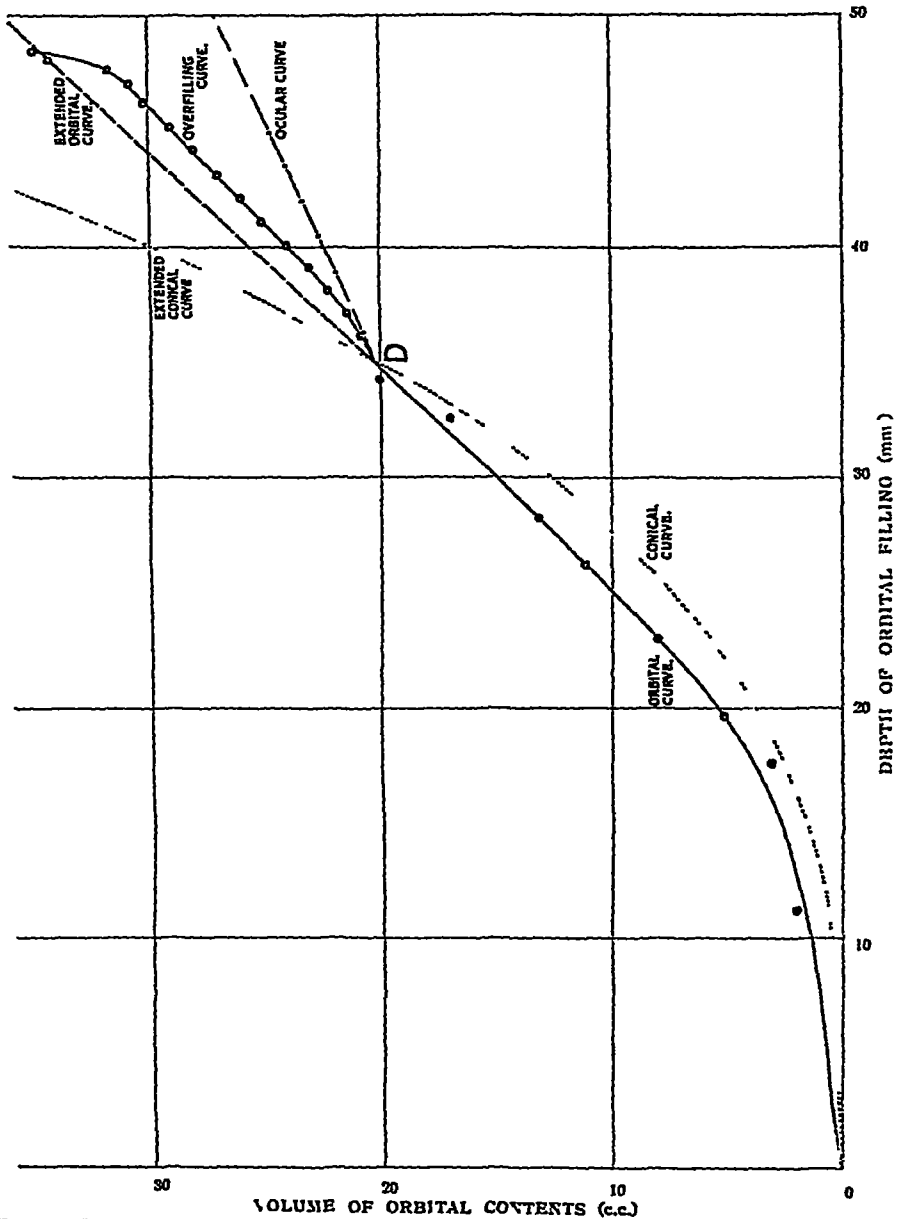
Fig 4 Comparison of different modes of orbital overfilling Protrusion of the anterior surface of the contents (mm.) is plotted against increase in their volume (c c) The curve of proptosis obtained in the experiment and other theoretical curves are superimposed on the depth filling curve of the orbit, at the point D which marks the actual filling prior to injections

It is evident that the gradient of the overfilling curve differs sharply from those of the ocular and extended conical curves but is roughly parallel, over most of its range, with that of the extended orbital curve Since the latter corresponds to a cylindroid of the same sectional area as the forepart of the orbit we may conclude that, for most of the overfilling range, the whole anterior face of the orbital contents, and therefore both the globe and lids are protruding *pari passu* Departures from the general parallelism between these two curves occur in the early and late parts of the range The more gradual gradient at the beginning of the injections corresponds with a smaller area of protrusion This might result from some tapering of the orbital cavity beyond the plane D but this seems unlikely for the altered gradient is maintained for only a few millimetres before the former gradient is regained A more likely explanation appears to be that the globe and tissues immediately round it at first yield more readily than those more peripherally placed But clearly the sectional area of protrusion never contracted to that of the globe alone

The terminal sharp increase in the gradient of the overfilling curve is very striking Proptosis is maximal and increases little in spite of further injections This may result from maximal extension of the optic nerve, the additional wax spreading forwards round the globe and centrifugally into the lids , this and overflowing of the orbital margins was verified at dissections

Referring again to Fig 2 we see that the shape of the several overfilling curves is similar When each was compared with the corresponding extended orbital curve a similar result was obtained * Hence we may conclude that when the bulk of the orbital contents increases protrusion occurs over the whole of the available sectional area of the forepart of the orbit Early reduction in area of the protruding section and late expansion are characteristic tendencies under the experimental conditions

Clinical observations on orbital overfilling

Exophthalmos may present puzzling problems to the clinician and it is clear that indulgence in general speculation as to its causation, especially in Graves' disease, has been responsible for misleading theories concerning the local pathology The simple mechanistic basis demonstrated for this sign in Graves' disease (4) and the results of injecting wax described here serve to focus attention sharply on the basic physical disturbance namely overfilling of the orbit It is clear that even in the slightest degree this must cause some exophthalmos

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* The overfilling curves from 10 different subjects (4 more than shown in Fig 2) were examined with this result But in one case (curve 2, Fig 2) the initial rate of protrusion was very rapid and for the first 5 6 mm , the overfilling curve corresponded approximately with the ocular curve The reverse was never found namely that the gradient of the overfilling curve at first exceeded that of the extended orbital curve Thus it is unlikely that protrusion of the lids ever precedes protrusion of the globe

Disparity between the bulk of the contents and capacity of the orbit, may, of course arise from a wide variety of clinical conditions but some expression such as orbital overfilling, overcrowding or disproportion is necessary to describe their basic and common pathology   In its strict sense exophthalmos is only one of the consequent signs   Lid protrusion is certainly another   It remains for clinical investigation to determine others and so to decide which of the manifold eye signs described in Graves' disease are explicable on this simple basis   Unfortunately " exophthalmos " is often used in an omnibus sense to describe all such cognate signs

Application to clinical diagnosis   It is useful to examine the plane of the lids with the eyes lightly closed   The general impression of exophthalmos depends on an increased extent of the globe's surface being exposed in the palpebral fissure   In simple retraction of the upper lid in Graves' disease, or of both lids in conditions of sympathetic excitation, an illusion of considerable exophthalmos may be created   But when such cases are examined with the eyes lightly closed such fallacies are avoided , the absence of bulging may enable the presence of any appreciable degree of overfilling or exophthalmos to be excluded at once

On the other hand positive evidence of lid protrusion may determine the diagnosis of overfilling and exophthalmos as in the following case —

Case 1   A man aged 74, found subsequently to be suffering from a small tumour of the R orbit, complained of double-vision and supra-orbital pain (Fig 5)   The exophthalmometer reading (Hertel) was R 18 0 ,   L 13 75 mm

With the head in the normal anatomical position and the gaze directed forwards the upper lid on the right side was slightly full , the plane of the lower lid was sensibly normal , there was no evidence of paralysis of the levator palpebræ or orbicularis oculi   But on the left side the upper lid was markedly sunken , the suprabulbar groove extended far up under the superior orbital margin, its depth, measured from the orbital margin, varying between 9-14 mm at different points   The left lower lid was slightly sunken

Proptosis of 4 25 mm was thus associated with a comparable degree of lid protrusion   In normal subjects the readings for the two eyes may differ by 3 0 mm or more but the lids are symmetrical   Thus in a series of 89 control subjects a difference of 2 mm was exceeded only once, the value being 2 75 mm , there was no associated asymmetry in the plane of the lids with the eyes lightly closed   Such differences in controls may depend more upon local anatomical factors affecting the baseline upon which the instrument rests, or the shape of the eyeball, than upon equivalent differences in the degree of filling of the two orbits   Without confirmation from asymmetry of the lids the diagnosis of overfilling and exophthalmos in this case would have remained doubtful

Finally it is instructive to consider the right eye in another patient (Figs 7 and 8)   Here extreme overfilling of the orbit and exophthalmos could have been deduced even though the usual clinical criteria of the latter were lacking   It was actually known (from serial measurements) that the right eye was proptosed by at least 6 5 mm but there was no undue widening of the fissure on this side and no band of sclera visible between the lower lid margin and limbus , in fact the lid margin covered ½ mm cornea * The exophthalmometer reading, 22 75 mm , though well above the average measurement in controls is not absolutely abnormal, the upper limit of normal being at least 23 0 mm   But clearly from the manifest extreme protrusion of the lids a severe degree of overfilling and exophthalmos could also have been inferred

CONCLUSIONS

Variations in the plane of the eyelids, sunken, normal or bulged, reflect corresponding variations in the degree of orbital filling as do correlative changes in position of the eye   The clinical characters of sunken and bulged lids are well-defined and, together with the exophthalmometer reading, enable departures from the average degree of filling to be detected

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* Slight retraction of the upper lid is present and accounts for any widening of the fissure This is of no significance in the diagnosis of exophthalmos but may contribute to the relative elevation of the lower lid

Fig 5 (*Case* 1) On the left, the lids are sunken on the right slightly full Exophthalmometer reading R 18 0 mm L 13 75 mm

Fig 6 Considerable bulging of the lower lid Note its lumpy character and peripheral distribution Orbital fat overlaps the malar bone

Fig. 4. (Same patient.) The right lower lid is also considerably protruded and tense but apart from the effect of slight lid retraction the right palpebral fissure is not widened.

Fig. 10. (Same patient. 7 months later.) All four lids are considerably bulged. [Exophthalmometer reading R. 27·25 mm. L. 27·75 mm.]
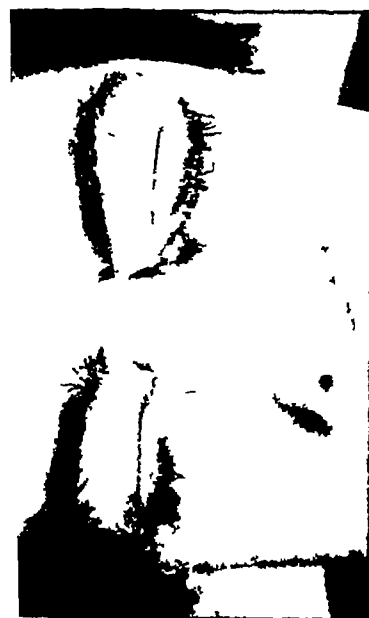
Fig. 7. Overfilling of the orbits and considerable bulging of the supra-tarsal part of the upper lids.

Fig. 9. Moderate fullness of the upper lids only. [Exophthal-mometer reading R. 21·75 mm. L. 21·5 mm.]

Fig. 12 — Effect of depression on protrusion of the lower lid

Fig. 11 — Effect of elevation on protrusion of the lower lid

THE ORBITAL TISSUES IN THYROTOXICOSIS

A QUANTITATIVE ANALYSIS RELATING TO

EXOPHTHALMOS *

By F F RUNDLE and E E POCHIN

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THE orbital contents have been examined in a series of 17 thyrotoxic cases and compared with controls † The average weight of the orbital tissues is found to be increased in the thyrotoxic group owing largely to an increase in fat content The increased bulk and fat content are correlated with the degree of exophthalmos present Certain changes in the composition of the tissues are found to be characteristic of thyrotoxicosis whether eye signs have been present clinically or not

Post-mortem recession of the eye

In normal subjects the eye recedes after death by about 2 5 mm with the onset of rigor mortis ‡ Measurements in thyrotoxic cases with exophthalmos show recession to occur but it is no greater than in normals (Table I) This persistence of exophthalmos after death indicates that the decisive factor in its maintenance cannot be a locally raised vascular pressure and is unlikely to be muscular spasm Previous reports as to the behaviour of exophthalmos after death are conflicting and unsupported by measurement It is clear from Table I that even measurements, if on single cases, might be misleading It is likely in addition that lid retraction may in some instances have been confused with exophthalmos clinically , its disappearance after death would then abolish the illusion of exophthalmos that it had created

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‡ In normal (1 case) and thyrotoxic (1 case) subjects it was noted that there was no recession immediately after death but that subsequently when the body was cold and rigor mortis well established recession had occurred as usual (Table I)

TABLF I

Differences in exophthalmometer reading before and after death

Readings in mm   negative values signifying post mortem recession of the eye   Fach line represents one subject

	R Eye	L Eye	Mean
Thyrotoxic cases with exophthalmos	—3 5	—3 25	—3 4
	—3 0	—3 0	—3 0
	—3 0	—3 0	—3 0
	—4 25	—4 25	—4 25
	—2 0	+0 25	—0 9
	—2 25	—3 25	—2 75
	—2 5	—3 0	—2 75

    Mean value —2 86   Standard error of mean ± 0 38

	R Eye	L Eye	Mean
Thyrotoxic cases without exophthalmos	—4 0	—2 75	—3 4
	—1 75	—1 5	—1 6
	—2 5	—1 25	—1 9
	—2 25	—2 75	—2 5
	—3 0	—2 0	—2 5

    Mean value —2 38   Standard error of mean ± 0 31

	R Eye	L Eye	Mean
Control cases	—2 0	—2 0	—2 0
	—2 25	—2 0	—2 1
	—4 0	—1 75	—2 9
	—5 75	—4 5	—5 1
	—3 5	—2 25	—2 9
	—0 25	—0 25	—0 25
	—4 25	—3 0	—3 6
	—5 5	—6 5	—6 0
	—2 5	—3 5	—3 0
	—2 5	0 0	—1 2
	+0 5	—	+0 5
	—2 0	—2 0	—2 0
	—2 5	—2 5	—2 5
	—2 0	—	—2 0

    Mean Value —2 50   Standard error of mean ± 0 45

The mean values do not differ significantly

It is evident that, if the position of the eye after death is determined by the bulk of orbital tissue behind it, this bulk must be increased in exophthalmos   That this is so is suggested by the persistence after death of marked protrusion of the lids in 5 exophthalmic cases of the present series, in which the sign had been present during life   -

Characteristic swellings above and below the eyes are frequently observed in thyrotoxicosis and have often been wrongly ascribed to oedema of the eyelids   The true nature of the swellings was suggested by Mr Wilfred Trotter on clinical grounds to be rolls of fatty tissue   This was confirmed at an operation done for cosmetic reasons on a patient with typical large swellings, rolls of fatty tissue were found bulging forwards out of the orbital cavity above and below each eye   The orbital origin of such swellings has been confirmed in other patients by a simple test If the lower swelling is pressed backwards towards the orbit the upper swelling increases and vice versa   If both swellings are pressed back simultaneously the eye moves forwards

Method of examining orbital tissues

The entire orbital contents from lids to apex of orbit were dissected and removed in a clean sweep   The volume of the orbital cavity was then measured   The various tissues were weighed fresh, after drying and after subsequent extraction with ether   Thus the fresh weight of any structure or its aqueous, fatty, and dry fat-free fractions could be determined and expressed in proportion to the orbital volume *

Dissection technique   The eyelids limit the orbital contents anteriorly   working first from in front they were raised from the subjacent tissues in the following way   The conjunctiva was divided along the upper margin of the superior tarsal plate and the cutaneous insertion of the levator palpebrae severed   By introducing the points of dissecting scissors in different directions and separating the blades the lid was now raised in the plane of its deep fascia, the orbital septum, as far up as the supra-orbital ridge   The lower lid was similarly freed from the peri bulbar tissues until the inferior orbital margin was plainly identified   The medial and lateral palpebral ligaments were then severed close to the bone

Working from inside the skull the roof of the orbit was removed as far back as the apex, taking care, however to preserve the medial and postero-lateral walls intact   The periorbita were incised longitudinally and the frontal nerve resected and discarded   The levator palpebrae superior rectus and superior oblique muscles were next dissected from their beds   cleaned and divided anteriorly at their musculo tendinous junctions in each case, and posteriorly at the extreme apex of the orbit   The lachrymal gland was then cleaned and removed.   Often it merged insensibly into the surrounding fibro fatty tissue, tissue of doubtful nature was left in the residue   A snippet of orbital fat was then taken from within the muscle cone

The trochlea was next detached and the soft tissues separated from the orbital margins and anterior parts of the bony walls   The eyeball was then displaced upwards and backwards and the inferior oblique divided at its bony attachment   The globe and its adnexa were further raised and the periorbita stripped up as far back as the apex   here the optic nerve and muscle attachments were divided.   The dissection was completed on a slab, the medial rectus inferior oblique inferior and lateral recti were cleaned and removed in that order   The optic nerve and eyeball were dissected from orbital fat and Tenon s capsule   All the latter fibro fatty tissue formed the residue

The orbital volume was measured by plugging the cavity with plasticine   The anterior face of the plasticine was slightly curved so as to run from the superior to the inferior orbital margin through the horizontal line joining the midpoints of the medial and lateral margins of the orbital outlet   Each orbit was filled three times   the volumes of the masses were obtained by weighing in air and water and the mean of the three estimates taken as the volume for that orbit

The technique of dissection was modified in patients in whom permission to open the skull was not obtained   The whole removal had then to be done from in front   The recti and oblique muscles were divided at their bulbar attachments and the optic nerve severed some 5 mm behind the globe   The globe was removed   As before the upper and lower lids were raised in the plane of the orbital septum.   The medial and lateral palpebral ligaments the trochlea and inferior oblique were next divided close to bone and the surrounding soft tissues with the periorbita separated from the orbital margins and walls back to the apex   Then retracting the tissues fully to the inner side the optic nerve and muscles were divided flush with the apex using sharp curved scissors   The specimen was then dissected on a slab   Measurement of the volume of the orbit was facilitated in these cases, plasticine was simply plugged in to fill it to the plane of the opening

Weighing and analysis   Each eye muscle, the lachrymal gland and the small sample (0 5 to 1 0 g) of orbital fatty tissue were received into small, weighed, stoppered bottles and weighed   The residual tissues (excluding the globe, optic and frontal nerves) were collected into a weighed bottle and weighed

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* Comparison of absolute weights is less helpful because of the wide range of variation in volume of orbits and because the weight of normal orbital structures varies with the volume of the orbit   The critical factor in exophthalmos is the ratio of the bulk of the tissues to the capacity of the orbit

All were then dried in a hot-air oven, thermostatically controlled at 108°C, cooled in a desiccator and then re-weighed The residual tissues are greatest in bulk and require 48 hours to dry, the remaining samples dry in about 4 hours It is always advisable, at least in the case of the residues, to verify the completeness of the drying by re-weighing after a further period in the oven

The dried tissues were then extracted with ether by the Soxhlet technique, the loss as fat being determined by weighing the extracted residues after drying in the oven to drive off ether It was possible to put through the six muscles, the lachrymal gland and fat sample from each orbit in a single batch by transferring the dried residues into numbered compartments of a filter paper folded into an eight-leaved rosette Extraction was complete in 2 hours The resulting dry fat-free residues are hard unbroken strips which can be transferred and weighed separately in a watch glass without difficulty Chloroform was used for extraction in certain earlier cases On comparing the amounts extracted from orbital tissues by chloroform and ether, no difference was found and these earlier results have been included with the others

Samples of other skeletal muscles and body fat were taken and analysed in the same way

Additional data The following additional data were also obtained —

Sex, age, diagnosis and cause of death

State at death obese, normal or little wasted, severely wasted, oedematous Controls were grouped in these four categories

Hertel exophthalmometer reading (post-mortem and sometimes ante mortem) This measurement gives the horizontal projection of the corneal apex beyond the lateral orbital margin, in mm

Samples were taken of infrahyoid muscle from just below the hyoid attachment, tongue from the midline near the dorsum, rectus abdominus, from the umbilical level, fat from the omentum or the abdominal wall

The specific gravity of the pooled orbital tissues was determined in a few cases by finding the volume of water required to fill a vessel, containing them, to a given mark An average value of 0 96 g per c c was found

In two cases a *detailed dissection* and analysis of the residual orbital tissues was carried out

RESULTS

The average volume of the orbital cavity is 26 c c , 70 per cent of which in normal controls is occupied by retro- and peri-bulbar structures The average weight of these structures is 18 9 g , which is made up of eye muscles, 3 3 g , lachrymal gland, 0 65 g and fibro-fatty residue, 15 g   Of the last about 12 g is recognisable fat, 2 5 g Tenon's capsule, 0 4 g nerves and

0 2 g blood vessels , but in the fresh specimen accurate dissection of these constituents is impossible and the residue has therefore been examined as a whole except in a very few cases

In the following sections the expression " orbital tissues " refers to all the orbital structures lying behind and round the eye excluding the optic and frontal nerves " Residual tissues " refer to those remaining after removal of the extrinsic muscles and lachrymal gland from the orbital tissues The component of a tissue extracted by ether is referred to as " fat " Since comparisons are primarily between particular findings in the thyrotoxic and control groups, differences within the control group due to wasting, oedema, age, and sex are discussed as they arise

Protrusion of the eye In normal controls the average post-mortem exophthalmometer reading was 16 0 mm but extreme values of 9 and 21 mm were observed In wasted controls the average value was lower and in obese subjects higher than in normal controls

The wide range in prominence of the eye among controls renders it impossible to state the amount of exophthalmos present in any thyrotoxic subject except when previous readings have been obtained on the same case Similarly, although marked exophthalmos is readily recognisable clinically, minor degrees fall within the range of normal variation and may be impossible to diagnose even when the effects of concomitant lid retraction are allowed for Thus no exact subdivision is possible but in the present series of 17 thyrotoxic cases —(a) 8 were regarded clinically as having no exophthalmos, (b) a further 5 were described as having exophthalmos but were not seen by us before death In view of the frequent confusion between lid retraction and exophthalmos the diagnoses in this group are uncertain but two had both high exophthalmometer readings and considerable protrusion of the lids post-mortem, and were probably exophthalmic (c) The remaining 4 cases were seen by us and were regarded as having exophthalmos in considerable degree and of typical appearance In one, serial exophthalmometer readings during life had demonstrated an increasing exophthalmos and the other 3 had swelling of the lids before and after death

The exophthalmometer readings are given in Table II

Although such readings may be unreliable in individual cases, the average values for the groups agree with the clinical diagnosis It may be assumed that certainly 4 and probably 6 of our thyrotoxic cases had typical exophthalmos to an average extent of about 4 mm *

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* This does not necessarily represent the frequency of exophthalmos in thyrotoxic subjects coming to post mortem since we were obtaining access to material from a number of sources and with special reference to exophthalmos

TABLE II

Exophthalmometer readings at post mortem in thyrotoxic and control cases (Readings in mm, average for both eyes)

Thyrotoxic cases

With no exophthalmos
16 0 14 5 16 6 14 2 18 5 16 9 15 8 16 0   *Mean value* 16 1

Said to have had exophthalmos
14 2 15 2 16 0 19 5* 21 6*   17 3

Seen by us to have had exophthalmos
19 2 19 2* 19 9* 21 6*   20 0

Control cases   Mean of whole group   17 3

	No of cases	Mean value	S E of mean	Standard deviation
Wasted	11	11 1	0 73	2 4
Normal	29	16 0	0 57	3 1
Obese	8	17 9	0 97	2 8

* Cases with protrusion of the lids

TABLE III

Relation between exophthalmometer reading and orbital filling

	Mean exoph reading (mm)	Mean orb filling (mg orb tissue per c c orb vol)	Correlation coeff within group	No of cases
Control cases				
Wasted	10 5	588	+0 23	10
Normal	16 1	689	}+0 69	25
Obese	17 6	760		7
All controls	15 0	677	+0 70	42
Thyrotoxic				
With no Ex	16 1	715		
Said to have Ex	17 3	744		
Seen to have Ex	20 0	808		
All thyrotoxics	17 3	744	+0 65	15

Degree of orbital filling Fig 1 illustrates the correlation between ocular prominence and the degree of filling of the orbital cavity in wasted, normal and obese controls and in thyrotoxic cases The post-mortem exophthalmometer readings are plotted against the ratio, weight of orbital tissues examined divided by total orbital volume Variations in the size of the globe and the specific gravity of the tissues are not taken into account by this method of presentation, but in spite of this, the correlation is evidentally close (Table III) A single curve relates differences in prominence among normal controls, the decreased prominence observed (Table III) in wasted cases and the increased prominence of obesity Similarly the data for thyrotoxic cases, with or without exophthalmos, lie on the same
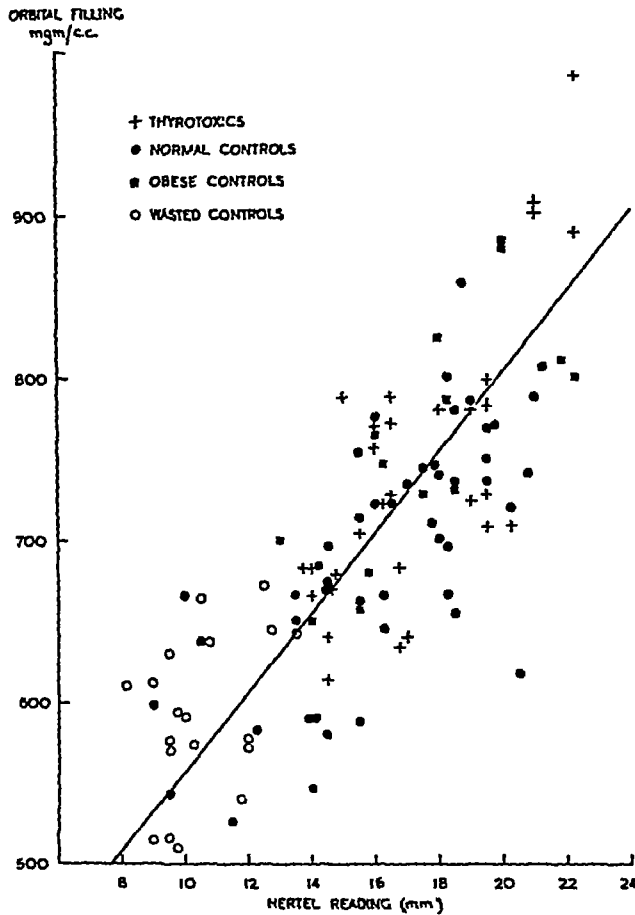
Fig 1   Hertel exophthalmometer reading in millimetres plotted against degree of orbital filling in mg orbital tissue per c c of orbital volume for thyrotoxic and control cases Each point represents one orbit

curve   It is quite clear that the position of the eye is determined by the amount of the orbital tissues and the extent to which they fill the orbital cavity, and that exophthalmos is caused simply by an increase in this filling

The distribution of the points in Fig 1 is approximately linear   The slope of this line shows that protrusion of the eye by 1 mm results from an increase in the bulk of orbital tissue by approximately 0 67 c c , assuming a specific gravity of 0 96 and a mean orbital volume of 26 1 c c   The cross-sectional area of the globe is about 4 5 sq cm   If the globe only were displaced forward, like a piston, 1 mm of protrusion should result from a 0 45 c c bulk increase   The difference between these figures is probably due to the simultaneous displacement forward of tissue round the eye as

the orbit fills, which can be seen to occur in many exophthalmic cases It follows from the figures observed that even a considerable exophthalmos of 6 mm would be caused by a 4 c c bulk increase which represents only 20% of the normal volume of the orbital tissues , and the average protrusion of 1 2 mm observed in the present series of thyrotoxic cases would result from a 4% increase in orbital filling This conclusion that considerable exophthalmos may follow small changes in volume is supported by the dramatic transient proptosis caused by retro-bulbar injection of small amounts of local anaesthetic solutions

Cause of increased orbital filling

Constitution of the orbital tissues The constitution of the orbital tissues in different groups of subjects is compared in Table IV In normal controls, fat forms about half the total weight of these tissues, occurring mainly in the residual tissues but also in muscles and lachrymal gland In wasting, the decreased values for residual tissue fat, and to a lesser extent muscle fat, show that the decreased orbital filling is associated with a loss of fat from these sites In obesity, despite an increased orbital filling, the constitution of the tissues is almost unchanged, the increase affecting both fat and fat-free components

TABLE IV

Constitution of orbital tissues (percentages)

| | Muscles | | Lachrymal gland | | Residual tissues | |
	Fat free	Fat	Fat free	Fat	Fat-free	Fat
Control cases						
Wasted	18 8	1 22	3 6	0 43	46 9	29 3
Normal	16 2	1 46	3 3	0 47	35 1	43 7
Obese	16 4	1 38	3 0	0 42	35 1	44 1
All controls	16 8	1 40	3 3	0 46	36 4	42 1
Thyrotoxic cases						
With no Ex	16 3	2 58	3 8	0 73	30 2	46 4
Said to have Ex	16 2	2 80	3 4	0 54	29 8	47 2
Seen to have Ex	16 8	2 59	4 2	0 55	30 0	45 3
All thyrotoxics	16 4	2 67	3 8	0 63	30 1	46 3

TABLE V

Constitution of orbital tissues (percentages)

| | Muscles | | Lachrymal gland | | Residual tissues | |
	Fat free	Fat	Fat free	Fat	Fat free	Fat
Normal controls						
males	16 6	1 22	3 3	0 42	35 4	42 4
females	15 0	1 54	3 3	0 61	34 6	46 0
Thyrotoxic cases						
males	16 8	3 30	3 6	0 72	27 4	47 7
females	16 3	2 53	3 8	0 61	30 6	46 0
Probability that differences between normal and thyrotoxic values are due to chance P=						
males	0 84	<0 01	0 64	0 02	0 02	0 18
females	0 08	<0 01	0 20	0 98	<0 01	0 99

TABLE VI

Weight of orbital tissues (mg per c.c of orbital volume)

	Muscles		Lachrymal gland		Residual tissues	
	Fat free	Fat	Fat-free	Fat	Fat-free	Fat
Normal controls						
males	109	9	25	3 4 .	240	287
females	109	11	22	3 3	241	334
Obese controls	123	11	20	3 3	260	355
Thyrotoxic cases						
males	134	20	28	5 6	226	395
females	116	18	29	4 6	226	338

In thyrotoxicosis with or without eye signs, the most conspicuous change is the considerable relative increase in muscle fat, its contribution to the orbital tissue weight is almost doubled   Fig 2 shows that in control cases, muscle fat forms a constant proportion of the orbital tissues for different degrees of orbital filling, and that the proportion is increased in thyrotoxicosis at all degrees of orbital filling   The figures for residual
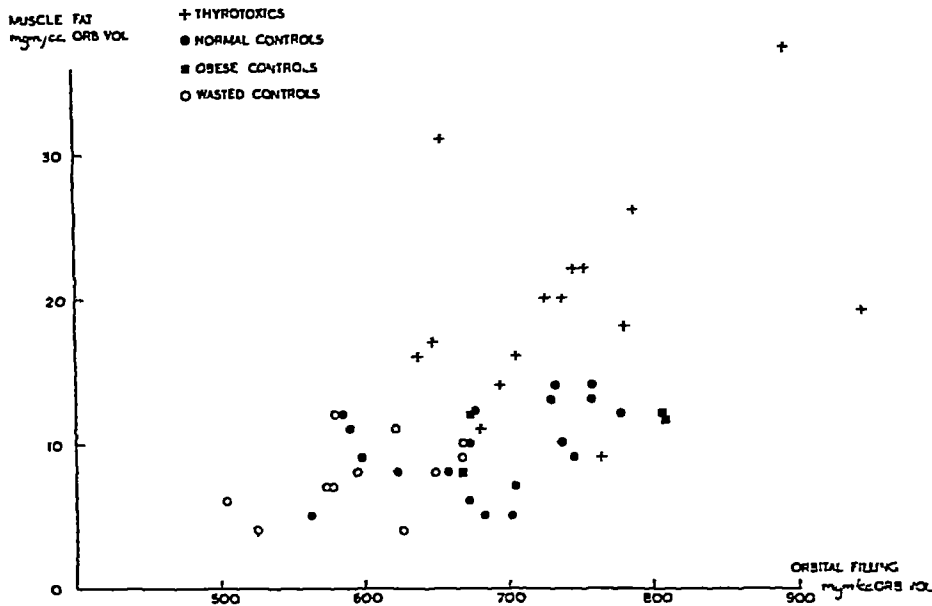
Fig 2   Amount of fat in the eye muscles, in mg per c c of orbital volume plotted against degree of orbital filling in mg orbital tissue per c c of orbital volume for thyrotoxic and control cases   Each point is the mean for both orbits in one subject

tissue and lachrymal gland fat are also increased though relatively less   A stricter comparison is made in Table V, in which the data for thyrotoxic males and females are compared with groups of normal controls of the same sex   This comparison is necessary since the fat contents of orbital structures

are normally slightly higher in females than in males, and since most of the thyrotoxic cases were females It will be seen that the increases in muscle fat are statistically highly significant * The relative proportion of orbital tissue formed by fat-free residue falls The corresponding absolute changes may now be considered

Weights of the orbital structures The average weights of orbital structures are less precisely determined than the relative constitution of the tissues This is because, in normal subjects, the weights of these structures vary widely in different individuals but, since all structures vary in proportion, the constitution of the total tissues is relatively constant Table VI gives, for different groups of subjects, the mean weights of structures when expressed in ratio to the orbital volume The use of this ratio decreases the variability of the data, since orbital structures vary in weight with orbital volume , it also measures orbital filling, which has been shown to be directly relevant to the degree of exophthalmos The greater changes in males presumably arise because all 3 male thyrotoxic cases were probably exophthalmic, whereas only 3 out of 14 females were so

Table VII gives in detail the increases in weight of orbital structures in the thyrotoxic subjects with high exophthalmometer readings In each case, the figures are based on the normal value appropriate to the sex of the subjects The same table also gives the average increase for the thyrotoxic group as a whole The average total increases in the exophthalmic and

TABLE VII

Increases in weight of orbital structures

Excess weights, over that appropriate for the sex, in exophthalmic thyrotoxic subjects (mg per c c orbital volume)

Case	Sex	Mean Hertel (mm)	Muscles Fat free	Muscles Fat	Lachrymal gland Fat free	Lachrymal gland Fat	Residual tissues Fat free	Residual tissues Fat	Total
G	M	19 5	+13	+13	+ 3	+1 8	+42	+ 55	⊥ 73
J	M	20 0	+29	+13	+ 7	+2 6	—26	⊥ 1	+ 81
L	F	19 2	+10	+ 9	+10	+0 7	—25	+114	+ 6
P	M	21 6	+32	+26	+ 2	+2 3	— 3	+ 85	+173
Q	F	21 6	+40	+ 8	+26	+0 6	+62		+222
Mean increase			+25	+14	+10	+1 6	(+2)	(+64)	+111
% increase			+23	+138	+40	+47	+ 1	+21	+16
P for group			<0 01	<0 01	0 06	0 06	0 80	0 13	<0 01
Significant change†			30	7	13	3	60	180	76
All thyrotoxic cases mean increase			+11	+ 9	+ 6	+1 5	—15	+22	+34
P for group			0 02	<0 01	0 10	0 06	0 60	0 15	0 07

* The values of P in this and subsequent tables indicate the probability, on t tests for the difference of means, that the differences could have arisen by chance Thus, P = 0 05 implies a 1 in 20 chance that the result is insignificant, and a 95% chance of significance

† This figure gives the magnitude of change which can be regarded individually as significant, with an 0 05 likelihood of being due to chance Case B is not included since the orbital volume was not determined The value of P is the probability that the difference is due to chance

TABLE VIII

Fat content of orbital structures (percentages of fat)

		Muscles	Lachrymal gland	Residual tissues
Control cases				
Wasted		6 9	9 6	37 9
Normal	male	7 7	11 2	53 8
	female	9 3	13 1	56 0
	all cases	8 1	11 7	54 5
Obese		8 0	13 0	56 0
Thyrotoxic cases				
male		16 5	16 5	63 5
female		13 2	12 9	60-0
all cases		13 8	13 5	60 5
Probability that differences are due to chance—				
Thyrotoxic and normal controls				
males		< 01	03	07
females		< 01	92	< 01
Thyrotoxic and obese controls				
all cases		< 01	82	09

the whole thyrotoxic groups are 112 and 34 mg per c c , which agree closely with the values of 108 and 30 mg per c c that would account, on the data of Fig 1, for the observed mean exophthalmos of 4 2 and 1 2 mm in these groups   In the exophthalmic group, increase of fat accounts for 71% of the change, that in residual tissues being responsible for 54%   Smaller increases in the fat-free component of muscles and lachrymal gland account for the remainder   The changes in muscle are statistically, highly significant, and those in lachrymal gland probably so

In proportion to normal values, the fat of each structure is increased more than the fat-free component   Muscle fat is most increased, being more than doubled, and this change is of high statistical significance, and the muscle fat has been significantly raised in each exophthalmic subject   Since, however, muscle fat normally forms only a small proportion of the total orbital tissues, its increase plays a small part in the total increase of bulk   On the other hand, the residual tissue fat is only increased by 20%, and the change is established with less certainty   In view of the large amount of such fat normally present, this increase is responsible for a large part of the total bulk increase

In the thyrotoxic group as a whole, the changes are smaller but of similar distribution, except that the contribution from the residual tissues is small   It seems likely that involvement of the muscles is a relatively frequent or early occurrence, but that the orbital tissues as a whole are not sufficiently increased in bulk to cause clinical exophthalmos unless the residual tissues are also involved   It may be noted that the muscle fat was increased in all but one thyrotoxic subject and significantly increased in 9 (and see Fig 6)

Composition of individual orbital structures   Further evidence on the change occurring in thyrotoxicosis is obtained from analysis of the orbital structures   The alterations in fat content which result from the changes already discussed are shown in Table VIII

The percentage of fat in the residual tissues, as well as in the muscles, is here shown to be significantly increased, and the changes exceed those in obesity, in which the muscle fat is unaffected

TABLE IX

Water content of fat free components of orbital structures (weight of water as percentage of fat free weight of structure)

	Muscles	*Lachrymal gland*	*Residual tissues*
Control cases			
Wasted	84 7	84 1	81 1
Normal	83 8	82 8	78 0
Obese	83 4	80 9	79 4
Thyrotoxic cases	83 1	82 4	74 0
These figures exclude one thyrotoxic case with abnormally low values —	75 2	74 6	68 1

This subject died after gastrectomy and the low values may possibly be due to dehydration

The composition of the fat-free components of orbital structures has not been studied except in regard to water content, which is unaltered in thyrotoxicosis, as shown by Table IX

It is thus established that in thyrotoxicosis and particularly in exophthalmos the fat content of the muscles and probably that of the lachrymal gland are increased absolutely, while that of the residual tissues is raised relatively and probably also absolutely   Smaller changes occur in fat-free tissue   The total change accounts for the observed alteration in position of the eye and the development of exophthalmos   These changes are not due to the general state of nutrition at death since obesity does not cause such increases of orbital fat, and as a group the thyrotoxic subjects were in fact wasted, in which condition a loss of fat normally occurs

The nature of the fat increase in residual structures and in muscles may now be considered in further detail

Distribution of pathological changes

Distribution of fat in residual structures   Of the fat normally contained in the residual structures about one-third is associated with Tenon's capsule, small amounts occur in nerves and vessels and the remainder is in recognisable adipose tissue   The percentage of fat in samples of this adipose tissue was not significantly altered in the thyrotoxic group   The water content of this tissue is also normal   This is shown in Fig 3 in which the values in thyrotoxics and normals are compared   Abdominal fat deposits in thyrotoxicosis were also normal in composition (Fig 4)
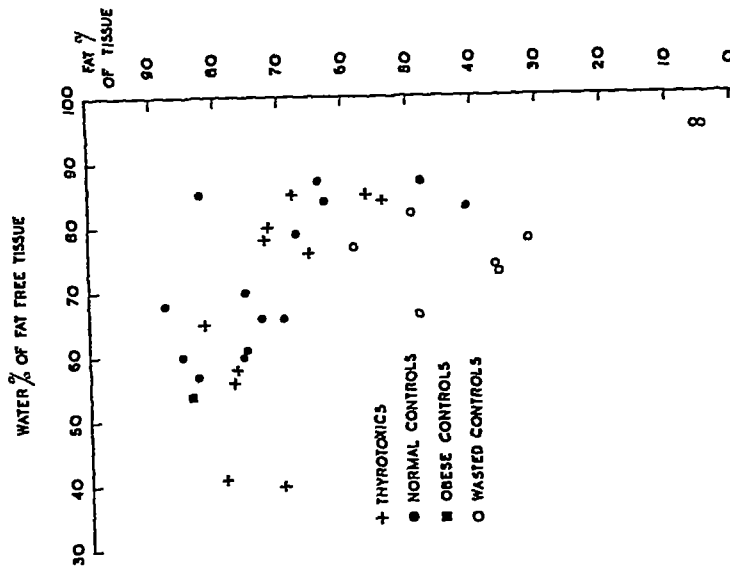
Fig 1 Adipose tissue from abdominal wall, relationship between water content and fat content, for thyrotoxic and normal subjects Each point represents one subject
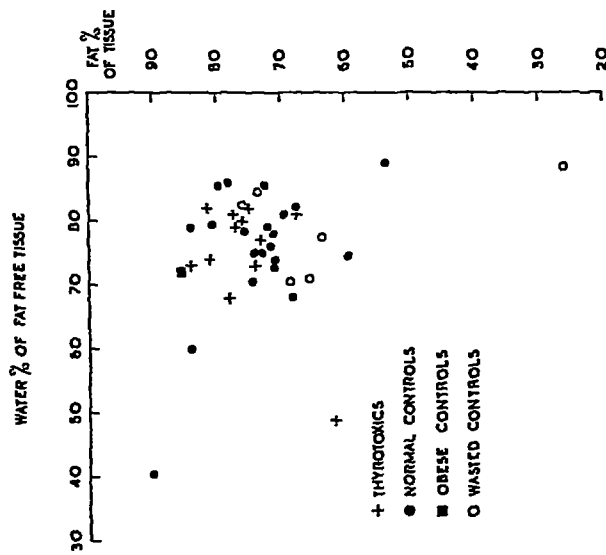
Fig 2 Orbital adipose tissue, relationship between water content and fat content for thyrotoxic and control cases Each point represents one subject, and is based upon samples from one or both orbits

It is found that the fat and water content of adipose tissue normally vary inversely, both in orbital and abdominal fat　In Figs 3 and 4 the fat percentage of the adipose tissue samples is plotted against the water percentage of the fat-free component　The results could be accounted for by assuming the loss in wasting of a water-free material containing 92% fat, from a fat-free structural material containing 95% water　The data for thyrotoxic and control subjects clearly lie on the same curve
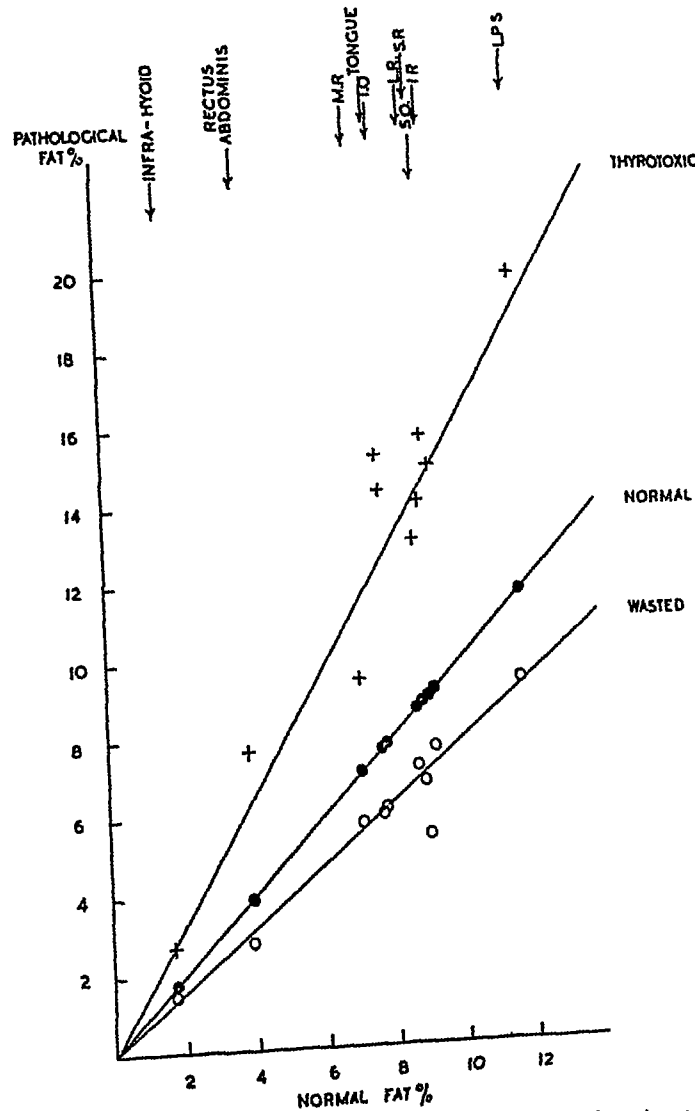
Fig　5　Fat content of eye muscles and other skeletal muscles in thyrotoxic and wasted cases, plotted against the normal fat content for each muscle　Each point is based on the mean value for the group of subjects　Lines are drawn to fit points for eye muscles　No obese control group is included since the individual ocular muscles were only analysed in two such cases
LPS, levator palpebræ superioris　SR, superior rectus　MR, medial rectus IR, inferior rectus　LR, lateral rectus　SO, superior oblique　IO, inferior oblique Tongue, sample from midline　Infra hyoid, sample from sterno hyoid　Rectus abdominis, sample from level of umbilicus

No examination has been made to determine whether the increased residual fat in thyrotoxicosis is due to an increase of normal adipose tissue or to an increased fat content of Tenon's capsule

The fat increase in ocular muscles When the individual eye muscles are analysed it is found that the increase of fat in thyrotoxicosis is not equal in different muscles but is proportional to the normal fat content of the muscle The levator palpebræ superioris, in which normally the fat content is highest, shows the greatest increase while the medial rectus, in which it is lowest, is least affected

In Fig 5 the pathological fat content is plotted against the normal fat percentage for that muscle and it is clear that the fat content is increased by about 60 per cent *of its normal value* in all muscles and not by an equal absolute amount in different muscles The fat percentage of the total mass of eye muscles was 8 1% in normal controls, and 13 8% in thyrotoxic cases In wasted controls it was 6 9% and in obese controls 8 0%

Conversely a decrease proportional to the normal value is observed in wasted subjects These facts suggest an abnormal metabolism of normal fat in thyrotoxicosis rather than an infiltration by abnormal fat No change occurs in the water content of the fat-free muscles, which is equal in different muscles, and in normals and thyrotoxics

Changes in other skeletal muscles In view of the changes in the ocular muscles other skeletal muscles were examined In controls, mean values of fat percentage are increased in obesity and decreased in wasting and the change is proportional to the normal fat content of the muscle (Fig 5) In obese controls, the fat percentages were infrahyoid 2 7%, rectus abdominis 7 7%, tongue 15 3%.

In thyrotoxicosis the fat content of the skeletal muscles is also increased but the values do not exceed those in obesity Thus, owing to the wide range of variation in controls, the result in thyrotoxicosis is inconclusive But since 11 of the thyrotoxic patients were wasted, 4 severely, the high skeletal fat content is probably not without pathological significance

Control observations

The thyrotoxic group consisted of 13 females and 4 males, the average age was 43 years The full series of normal controls in whom the orbit was examined consisted of 16 females and 32 males, the average age was 48 years In view of these differences between the samples, the effect of age and sex in normal controls has required examination

Effects of sex Mean values for orbital structures in male and female normal controls are given in Table X The only significant difference observed is in the orbital volume, which is larger in males than in females by 12% * The degree of filling of the orbit does not differ significantly in

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* It may be noted that for this reason a survey of total orbital tissue weight in thyrotoxic subjects of which most are females could not be compared directly with a sample of normal controls of which half were males The 6% mean difference in orbital volume would mask orbital overfilling corresponding to an average exophthalmos of 1 7 mm

TABLE X

Sex differences in orbital structures

Mean values in normal controls

		Male	Female	P*
Exophthalmometer reading (mm)		16 0	16 1	0 76
Orbital volume (c c)		27 3	24 4	< 0 01
Orbital tissue weights (mg per c c of orbital volume)				
Muscles	fat free	109	109	0 99
	fat	9 1	10 7	0 28
Lachrymal gland	fat free	25	22	0 26
	fat	3 4	3 3	0 23
Residual tissues	fat free	240	241	0 97
	fat	287	334	0 20
	Total	673	720	0 53
Constitution of orbital tissues (per cent of total)				
Muscles	fat free	16 5	15 3	0 00
	fat	1 38	1 65	0 40
Lachrymal gland	fat-free	3 31	3 39	0 83
	fat	0 43	0 58	0 06
Residual tissues	fat free	35 4	34 6	0 68
	fat	42 6	46 0	0 12
Fat content of orbital structures (per cent)				
Muscles		7 7	9 3	0 18
Lachrymal gland		11 2	13 1	0 28
Residual tissues		53 8	56 0	0 55

* The value of P is the probability that the difference is due to chance

TABLE XI

Effects of age on orbital structures

		Correlation coefficient, with age	
Exophthalmometer reading	males	+0 04	P = large
	females	—0 06	P = large
Orbital volume	males	+0 26	P = large
	females	+0 26	P = large
Structure weights divided by orbital volume			
Muscles	fat-free	—0 39	P = 03
	fat	+0 61	P = < 01
Lachrymal gland	fat free	—0 29	P = large
	fat	—0 12	P = large
Residual tissues	fat free	+0 35	P = < 01
	fat	+0 92	P = < 01

The value of P is the probability that the correlation is due to chance　Values larger than 0 10 are given as large

Data based on normal and obese controls

the two sexes and the mean exophthalmometer readings are equal Although no clearly significant differences in composition of the orbital tissues is observed, the slightly higher fat contents of orbital structures in the female have been taken into account in comparisons between thyrotoxic and normal cases This is necessary in view of the predominance of females in the thyrotoxic series and of males in certain control series, and because the main changes in thyrotoxicosis consist of increased fat contents

Effects of age With increasing age the fat content of muscle and of residual tissue rises, while the weight of fat-free muscle probably falls (Table XI) The resultant rise of fat percentage in normal muscles is shown in Fig 6, where the data of normal and obese controls are plotted as discs The corresponding data of thyrotoxic cases are plotted as crosses, and show that the muscle fat in the subjects examined far exceeds that appropriate for their ages The average age of the thyrotoxic group is in fact less than that of the control group, so that the increases in fat content of muscles and residual tissues described in thyrotoxicosis slightly understate the true values
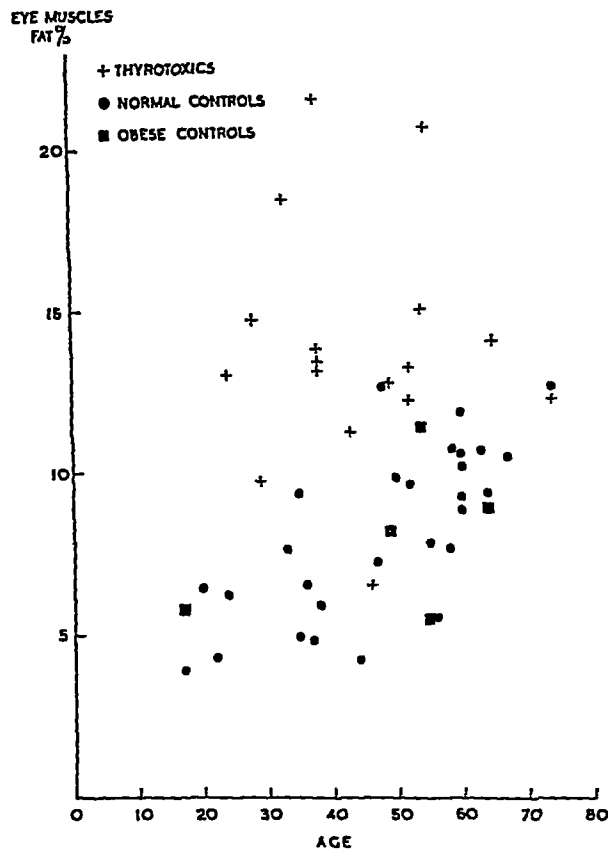
Fig 6 Eye muscle fat percentage plotted against age of subject for thyrotoxic and control cases Each point represents one subject being usually based on the mean for both orbits The percentage is that of total fat in all eye muscles to total weight of the muscles

Fig 6 also demonstrates the frequency of significant changes in muscle fat percentage in thyrotoxicosis, even in a group of cases of which the majority had no exophthalmos

Effects of oedema In four of the thyrotoxic subjects there were varying degrees of oedema of the lower half of the body post-mortem owing to terminal congestive cardiac failure The orbital findings were, however, the same in cases with and without oedema, and no comparable changes were found in a control group with gross oedema

Controls had oedema from cardiac failure in 3 cases and of renal origin in 2 cases Some of these cases were also wasted The average fat content of the ocular muscles was reduced to 6 8 per cent and the orbital filling to 664 mg per c c The water content of the fat-free component of the ocular muscles was increased from 83 8 per cent to an average of 86 5 per cent and that of lachrymal gland and residual tissues was probably slightly raised No other changes were noted except those characteristic of wasting

Effects of wasting No attempt has been made to control the thyrotoxic series against a series with an equal average degree of wasting Eleven of the 17 thyrotoxic subjects were wasted, 4 severely, while one was classified as obese despite the loss of 2 stones in 6 months Since this group has been compared with controls of normal nutrition or slight wasting, and since the changes observed have been opposite to those occurring in wasting, the extent and the significance of these changes in thyrotoxicosis have probably been substantially underestimated

Statistical methods Tests of the significance of differences of means have been made by the t test (1) This method is rigorous as a test of the null hypothesis that a variate is unaffected by the disease process in mean value, dispersion or distribution It is likely, however, that it is unduly stringent in comparisons between a group of normals and a group of thyrotoxics of whom many, where the disease process is mild, are virtually normal, thus detracting from the significance of the abnormal results in the few exophthalmic cases Behrens's test (2) has given similar results because it allows for the greater dispersion of the variate in the thyrotoxic group but not for the abnormal distribution

Every comparison has been made on all the data available, so that different comparisons are not necessarily based on identical series of cases This results in minor variations in mean values, and the results of different comparisons cannot be directly combined

DISCUSSION *

Nature of the pathological process The increased bulk of the orbital tissues occurring in thyrotoxicosis appears to be due mainly to an increase in the fat content of the orbital structures In view of the small changes sufficing to produce exophthalmos and the variability of normal tissues, it is difficult to analyse the change closely except in the extrinsic muscles where the percentage increase is considerable It is here found that the different muscles are affected in direct proportion to their normal fat content, as in the opposite changes of wasting, which suggests an abnormal regulation of normal fat rather than a generalized deposition of abnormal fat In the absence of evidence that fat migrates from the muscles to surrounding structures, it is likely that this abnormal regulation affects other sites of

* Seitchik (6) has recently reviewed (80 references) the conflicting evidence as to the mechanism of exophthalmos in Graves' disease Since earlier data have not been quantitative in character no attempt is made to discuss them here

orbital fat deposition   Further, the findings in other skeletal muscles suggest that abnormal regulation of fat may not be confined to the orbit, conditions in the orbit merely being such that an increase of bulk here produces striking results

The changes are the more impressive since they are observed in wasted subjects, although wasting in control cases without thyrotoxicosis is characterized by loss of fat from the orbit, mainly from residual tissues and to a less extent from the extrinsic muscles   At the same time the changes in thyrotoxicosis differ from and exceed those seen in obesity in which the orbital tissues are also increased   In obesity the increase involves material other than fat, so that the proportion of the different constituents is little affected   In addition the fat content of the eye muscles has not been increased in the cases examined, despite large increases in other skeletal muscles   In thyrotoxicosis, although the increase probably involves material other than fat, the increase in fat proponderates and disturbs the constitution of the orbital tissues   Also the fat in the eye muscles is increased as much or more than in the other skeletal muscles examined   It is also of interest to note that Case K, the only thyrotoxic classified as obese at death despite some loss of weight, had no exophthalmos clinically, low exophthalmometer readings and considerably the lowest extrinsic muscle fat percentage in the thyrotoxic group   It is quite clear that the changes observed in thyrotoxic cases are not simply due to concurrent obesity, but represent a deposition of fat which occurs despite general body wasting   Slight changes in the same direction occur in advancing age, and possibly to a greater extent in females

Relationship to clinical signs   In the group of thyrotoxic cases with typical exophthalmos, the estimated increase of orbital contents was sufficient to account for the degree of exophthalmos observed, and to explain in addition some protrusion of the tissues round the eye   It is clear that both these eye signs, exophthalmos and the swellings above and below the eye which may accompany it, become apparent clinically when the orbital tissues are increased by a certain amount   It is likely that in thyrotoxicosis this stage is only reached when the residual tissue fat becomes materially increased   The physical mechanism of protrusion of the globe and lids is examined in more detail elsewhere (5)

The increase in muscle fat may, however, be extensive before the residual tissue fat is sufficiently increased to cause exophthalmos   No clinical correlation has been attempted between disturbance of fat content and of muscle function in the present series, since too few cases were seen by us during life   It is significant, however, that the muscle most severely affected by the increase in fat, the levator palpebræ superioris, is also that whose shortening has been shown to cause the commonest of the eye signs, lid retraction (3)   Moreover, it has now been established on quantitative grounds that disorder of muscle function amounting to ophthalmoplegia is common in thyrotoxicosis (4)   It appears probable that lid retraction, ophthalmoplegias and possibly other eye signs may prove to be related in

TABLE XII

Clinical data from thyrotoxics

Thyrotoxic group Case	Sex	Age	Thyrotoxicosis, duration and severity	General nutrition and oedema	Eye signs present
A	F	40	Nodular toxic goitre with moderate thyrotoxicosis for 10 years, auric fib Pulmonary infarction Died from cardiac failure	Wasted Oedema of legs	"Slight stare"*
B	F	24	Thyrotoxicosis for 5 years, severe for the past 6 months	Normal No oedema	Considerable exophthalmos, lid retraction and lag both positive
C	F	38	Thyrotoxicosis for more than one year, auric fib Died from congestive failure with oedema	Severe wasting Ascites and oedema of lower half of body	No lid retraction or lag Exophthalmos not present
D	M	44	Mild thyrotoxicosis for 6 years, paroxysmal auric fib Also suffered from gastric ulcer, died after gastrectomy	Severe wasting No oedema	"Marked exophthalmos"
E	F	74	Goitre for 40 years Secondary thyrotoxicosis for more than one year, auric fib Died from congestive failure with oedema plus bronchopneumonia	Slight wasting Oedema of legs	"Exophthalmos present"
F	F	38	Thyrotoxicosis for 3 years, severe for the past 9 months Died in crisis	Wasted, had lost 3 stone No oedema	"Marked exophthalmos"
G	M	54	Severe thyrotoxicosis for 1 year, died in crisis following thyroidectomy	Severe wasting No oedema,	"Considerable exophthalmos and bulging of the lids Dalrymple's and von Graefe's signs also present" Post mortem there was well marked exophthalmos with obvious protrusion of the upper and lower lids by prolapsing orbital fat
H	F	33	Severe thyrotoxicosis for at least 6 months	Severe wasting No oedema	"Exophthalmos very marked Dalrymple s and von Graefe's signs present"
I	F	37	Thyrotoxicosis (mild) plus cerebral tumour the latter caused death in coma	Normal No oedema	Eyes prominent but no fulness of lids true exophthalmos not present
J	M	35	Severe thyrotoxicosis for 2 years died in toxic delirium	Moderate wasting No oedema	Lid retraction and lag (R) ophthalmoplegia of elevation (I) Moderate bulging of upper and lower lids (R and L) Hertel reading R 23 0 L 21 0+ Considerable exophthalmos present

	Sex	Age	Clinical history	Wasting / oedema	Eye signs
K	F	40	Thyrotoxicosis for 3 years becoming severe in the past 6 months Died in crisis following thyroidectomy	Had lost 2 st in the past 6 months but still very well nourished No oedema	No lid retraction or lag present Eye move ments full No fulness of lids Hertel reading R, 17 75, L, 17 5 Exophthalmos not present
L	F	25	Severe thyrotoxicosis for 8 months died in crisis	Moderate wasting, had lost 2½ st in the past 3 months No oedema	Slight paralysis of elevation (R and L) with lagging of the upper lids Hertel reading was observed to increase from R, 22 5 L, 22 5 to R, 23 75, L, 23 25 and moderate fulness of the upper lids to develop in the 9 weeks for which she was under observa tion During this period also the range of elevation decreased by about 10° and lagging of the upper lids developed Well marked exophthalmos present
M	F	20	Mild thyrotoxicosis, died in crisis after thyroidectomy	Normal No oedema	No lid retraction but slight lag present No definite fulness of upper or lower lids Hertel reading R, 10 0, L, 10 25 Exophthalmos not present
N	F	52	Goitre for 20 years, secondary thyrotoxi cosis for at least 6 months Died following thyroidectomy	Wasted (had lost 3 st in the past 6 months) No oedema	Slight ophthalmoplegia of elevation (R and L) No lid retraction or lag Lids not bulged Hertel reading R, 16 25, L, 16 0 Exophthalmos not present
O	F	52	Severe thyrotoxicosis for 6 months, died in crisis	Wasted No oedema	L eye amblyopic with marked divergent squint (old injury) R eye movements full, slight lid retraction and lag due to spasm of the levator palpebrae No fulness of the lids on either side Hertel reading R, 21 5, L, 20 0 No clear exophthalmos present
P	M	55	Severe thyrotoxicosis for 2 years auric fib Died following thyroidectomy	Wasted Had lost 1 st No oedema	"Conspicuous exophthalmos" Dalrymple s and von Graefe's signs also present Post mortem, considerable fulness of both upper lids, more marked on the L; definite fulness also of lower lids Hertel reading (post mortem) R 21 0 L 22 5 Considerable exophthalmos present
Q	F	13	(1) Mild thyrotoxicosis for 10 years (2) Hypertension (1) Thyasiul empty (1) Auric fib Died from cardiac failure with oedema	Normal Oedema of lower half of body	Considerable protrusion of the upper and lower lids on both sides Hertel readings (post mortem) R, 21 0 L, 22 5 Considerable exophthalmos present

* Eye signs given in quotation marks were recorded by others
† Unless otherwise stated, the exophthalmometer readings quoted in this table were made ante mortem

TABLE XIII

Quantitative data from thyrotoxic cases

In this Table " I " in column 6 indicates that the individual eye muscles were analysed
Hertel readings in column 3 are post mortem values
In all samples analysed water content was also estimated

Orbital tissues, thyrotoxic group

Case	Side	Hertel mm	O Vol c c	Muscles mg	I	L G mg	Resid mg	Mm mg	L G mg	Resid mg	Orb fat %
						Total weights			*Fat Contents*		
A	R	16	—	3483	—	484	12143	455	70	7010	—
	L	16	—	3533	—	591	11456	438	99	6430	—
B	R	18½	—	3014	I	546	11912	381	55	—	77 1
	L	20	—	3012	I	488	11989	404	78	—	—
C	R	14½	25 5	3667	I	771	11871	789	142	7182	84 3
	L	14½	23 2	3390	I	623	11528	730	127	7020	83 3
D	R	14	29 1	3929	I	470	15466	529	66	8337	65 0
	L	14½	31 0	3920	I	499	14595	496	71	8088	57 7
E	R	16¼	29 2	3598	I	1064	16417	436	96	—	60 2
	L	14	30 4	3260	I	1045	15538	441	120	—	74 4
F	R	15½	26 1	3541	I	861	13967	471	90	—	73 2
	L	16½	23 1	3422	I	779	13617	493	79	—	74 5
G	R	19½	31 6	4832	I	1263	18671	745	208	—	76 8
	L	19½	35 1	4694	I	920	19202	680	139	—	69 0
H	R	15	23 5	3285	I	713	14524	542	120	8316	76 4
	L	16¼	23 4	3325	I	921	14210	673	138	8461	78 4
I	R	18	23 7	3367	I	652	13896	457	100	8336	76 4
	L	19	23 6	3169	I	868	13913	413	132	8541	86 5
J	R	19½	26 7	4673	I	883	15714	696	142	9658	81 8
	L	20¼	29 4	4270	I	1289	15250	556	109	9502	82 3
K	R	16	26 6	3388	I	720	16053	211	80	9558	78 0
	L	16	25 5	3491	I	656	15494	236	52	9486	71 5
L	R	19½	24 1	3370	I	891	13300	473	99	8014	77 0
	L	19	23 8	3290	I	839	13075	493	94	8016	78 3
M	R	13¾	21 5	2468	I	949	12208	256	207	7773	—
	L	14¾	22 2	2375	I	855	11830	215	203	7798	—
N	R	16½	28 0	3329	I	895	14882	447	180	9148	—
	L	16½	26 7	3318	I	882	15228	429	192	9234	—
O	R	17	24 0	3157	I	910	11298	383	78	6906	—
	L	16¼	23 5	3077	I	837	10972	367	68	6759	—
P	R	21	23 8	4356	I	944	16145	931	191	10606	68 5
	L	22¼	24 6	4277	I	520	17149	855	82	10907	84 7
Q	R	21	25 1	4189	—	1370	17121	432	114	10036	—
	L	22¼	24 1	4110	—	1183	18359	496	79	10545	—

TABLE XIII—*continued*

Muscles and adipose tissue

Fat percentages

Case	Body fat	Infra hyoid	Rectus Abd	Tongue	Eye Muscles
A	62 6	—	—	—	12 7
B	70 0	—	—	—	13-0
C	68 0	—	—	—	21 5
D	65 5	—	—	—	13 1
E	54 0	2 9	6 8	11 0	12 2
F	82 1	1 9	3 7	15 0	13 8
G	72 7	1 9	7 8	21 2	15 0
H	72 4	3 3	6 2	17 7	18 4
I	—	4 7	8 8	15 3	13 3
J	77 0	3 2	13 7	10 7	14 0
K	79 3	1 3	9 8	9 8	6 5
L	77 7	3 6	11 2	11 6	14 7
M	—	2 0	8 5	22 0	9 7
N	—	3 4	7 3	6 7	13 2
O	—	1 9	9 5	16 6	12 2
P	—	1 8	1 6	24 8	20 6
Q	—	3 2	5 4	12 6	11 2
R	—	1 1	—	—	—
S	—	2 2	—	—	—
T	—	2 6	—	—	—

some way to the extensive increase in fat content of the extrinsic muscles in thyrotoxicosis

Fat changes similar to those found in patients with exophthalmos were present in the remaining cases of thyrotoxicosis although in less degree Thus even when eye signs were absent clinically there was often well-defined pathological change It appears that exophthalmos merely results from a high degree of a process common to all cases of thyrotoxicosis This clarifies the general concept of thyrotoxicosis, nosological attempts to separate " exophthalmic " from other types of toxic goitre tend to be controverted by the present data The same is true for the proposed segregation of patients with severe ocular changes into a different syndrome " malignant exophthalmos " Thus 3 of the present cases had been proved by measurement to have ophthalmoplegia, it may also have been present in others not examined clinically In at least 4 and probably 6 there was considerable exophthalmos In one case serial exophthalmometer readings had shown the orbital changes to be rapidly growing worse In 5 there was well marked lid protrusion Thus in this group of 17 thyrotoxic patients both ends of the clinical scale were well represented, that in which the eye signs were absent (8 cases) and that in which they were present in marked degree (4-6 cases) There was, however, no indication of any essential difference of the pathological process, except in degree, between the groups The decisive factor in the markedly exophthalmic cases was the high orbital-filling ratio There was no evidence that its mode of production was different in the high and low degrees Extreme protrusion of the globe

and lids, prolapse of conjunctiva and corneal ulceration presumably develop when the ratio, bulk of orbital tissues to orbital volume, progresses to a maximum   It would seem reasonable to accept the view that severe and mild exophthalmos in Graves' disease are similar in nature and that a common process is operating severely in the one case and mildly in the other

SUMMARY

Exophthalmos in Graves' disease is accounted for quantitatively by an increase in bulk of the retro-bulbar tissues

The increase is relatively greatest in the eye muscles, of which the average fat content was doubled in a series of 17 thyrotoxic cases   The changes are most marked in the levator palpebræ superioris muscle which is known to be responsible for causing lid retraction   Increase of fat in the orbital fibrofatty tissue is, however, responsible for most of the increase in bulk

The changes are present both in cases with and without eye signs

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EXOPHTHALMOS IN GUINEA PIGS INJECTED WITH PITUITARY EXTRACTS

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EXOPHTHALMOS in guinea pigs injected with pituitary extract was first reported by Loeb and Friedman in 1932 (2) and the same phenomenon has been described by subsequent workers (1, 3, 4, 5, 6, 9, 10) These reports, however, vary widely as to the circumstances in which exophthalmos is said to occur   Conflicting statements have been made on its frequency, how soon it develops whether it increases or regresses with continued injections, its persistence after death and its augmentation by thyroidectomy These discrepancies have clearly arisen for two reasons   Firstly, the prominence of the eye has usually not been measured and, as is described later, naked eye judgment as to the presence of exophthalmos is grossly inaccurate, even when based on paired animals   Secondly, in the cases where measurements of the prominence of the eyes have been made (6 and 10), the effects of changes in body weight itself on the position of the eyes have been ignored, so that the amount of exophthalmos has not in fact been determined

Exophthalmos in the guinea pig can however, be estimated accurately by a simple method involving two stages —determination of the animal's inter corneal distance , and estimation of the amount by which this exceeds that which would be normal in the animal

Method

Measurement of inter-corneal distance   Since the eyes project laterally, any protrusion will correspondingly increase the inter-corneal distance (I C D)   The amount of this I C D can readily be measured by adjusting a pair of parallel-bladed calipers so that each blade is aligned onto one corneal apex without, of course, touching it   Calipers with a rack and pinion adjustment and a vernier scale have been used   Readings are made in a warm, quiet room, preferably that in which the animal lives   The

animal is placed on a table facing away from the observer who rests his wrists on each side of the animal and aligns the blades with the corner, checking the alignment of each in turn   The method could be facilitated by a suitable optical device, but four readings to a tenth of a millimetre can be made in 1½ minutes, and give an average which is found on repetition to be accurate to within 1/4 mm , and this is the measure of I C D used in this paper   After a few readings even young animals remain quiet, particularly if their interest is maintained by suitable quiet hissing or whistling

Estimation of exophthalmos   In growing animals, the I C D increases regularly with body weight and, if daily values of one are plotted against the other, the successive points lie close to a straight line (Fig 1)   Moreover, if the body weight falls, for example on thyroxine administration, the loss of weight is associated with a corresponding reduction in I C D , so that the points now retrace the same or a closely similar line   It is therefore possible to speak of a value of the I C D appropriate to any given body weight in a particular animal   If now a pituitary extract is given, the I C D begins to exceed that appropriate to the body weight   I have estimated exophthalmos as the amount by which the I C D exceeds that which would normally correspond to the body weight of the animal under investigation

Before producing exophthalmos, therefore, the animal is measured daily as it grows from about 150 to 200 g in body weight   Plotting these daily measurements of I C D against weight, the points approximate to a straight line   The animal is now injected   If an inert solution is used, subsequent points continue this line (Fig 1a)   If thyroxine is injected, the points retrace the line or more commonly fall slightly below it (Fig 1b)   With suitable pituitary extracts, the points rise above the line (Fig 1c) indicating an I C D abnormally large for the body weight   The amount of exophthalmos on any day after injection is estimated simply as the height of the corresponding point above the base-line formed during growth   Figures are given in millimetres and refer to the combined protrusion of both eyes

Accuracy of estimation of exophthalmos   The accuracy with which exophthalmos can be detected or estimated by this method depends upon the degree of scatter of the preliminary data from the line relating I C D to weight   This scatter can be made small by attention to three factors —

1   Errors in measurement of I C D are small when the animal is quiet but, if it is startled, the eyes may be retracted for a short period and cause a fallacious reading   Other animals should not be feeding in the room during measurements, and the room should be warm and quiet   Albinos should not be used since they partially close their eyes in strong light

2   Unless weights and measurement are made at a fixed time of day preferably before feeding, the amount of the stomach contents affects the
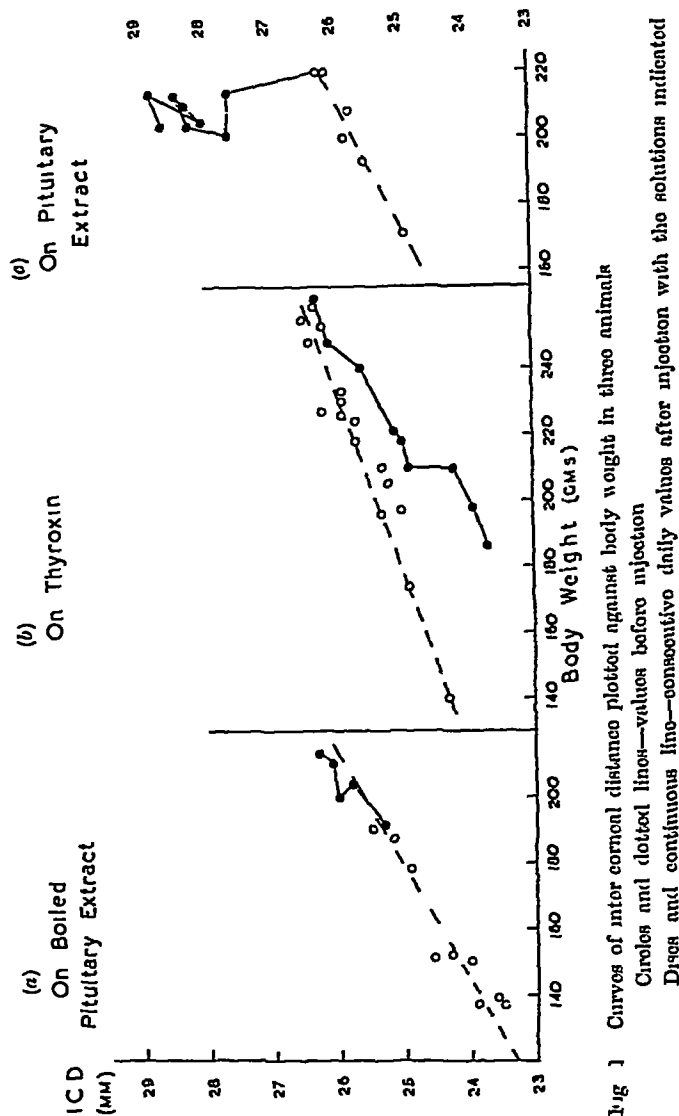
Fig 1 Curves of inter corneal distance plotted against body weight in three animals

Circles and dotted lines—values before injection

Dises and continuous line—consecutive daily values after injection with the solutions indicated

weight but not the I C D , and irregularity results   In the present work, animals had a single daily feed at 10 a m   after being weighed and measured

3   Although the I C D falls with loss of weight, it normally falls to a slightly lower value than corresponded to the same body weight during growth ,   and in some cases the decrease in I C D lags behind the loss in weight   Consequently, loss of weight from diarrhœa, cold, change of diet

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or recent travel should be avoided during the observations preliminary to the estimation of exophthalmos It is also important that, if different treatments are being compared, animals should not be grouped in cages according to treatment Otherwise an epidemic of diarrhœa in one cage may, by causing loss of weight, cause a reduced estimate of exophthalmos on the corresponding treatment

In animals which lose weight, exophthalmos is underestimated by about 0 25 mm per 10 g lost No attempt has been made to apply this relatively small correction in the present work, and it is preferable to avoid this loss of weight than to correct for it It has been observed that the loss of weight that results from 4 daily injections of pituitary extract (each containing 6 to 12 units of thyrotropic hormone) is so related to the initial body weight that the final body weight becomes about 180 grams (Fig 2) This odd result holds for initial body weights between 200 and 250 grams and implies that, in the strain used, exophthalmos is most reliably estimated by injecting the animal as it reaches about 200 grams
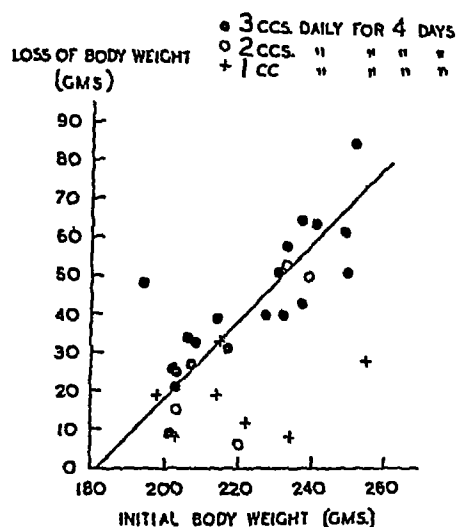
Fig 2   Loss of body weight in grams after four daily injections of pituitary extract, plotted against body weight of animal in grams when first injected

In well-fed animals growing steadily, exophthalmos can be estimated to within 0 5 mm by the method described   Thus, in 12 animals growing from 180 to 300 grams, 330 daily readings gave points which were displaced from the I C D curves of the animals with a standard deviation of 0 22 mm of I C D , only 13 readings being 0 5 mm or more off the appropriate line

Previous methods of detecting exophthalmos   Results obtained by the present method suggest that many of the discrepancies in previous reports may be ascribed to the methods used in detecting exophthalmos   Naked eye detection of " exophthalmos " appears to be based mainly on the absolute value of I C D or on correlated changes in palpebral fissure   During

the present work, laboratory workers were asked to arrange animals within groups of four in order of exophthalmos   Normal growing animals were very commonly judged to be exophthalmic, when compared with exophthalmic animals which had lost weight or ceased growth during pituitary injections, and the method is quite unreliable for either estimating or detecting exophthalmos   The objection applies to the method of Loeb and Friedman (2), who paired animals having eyes of similar appearance before injecting one member of each pair   The method could be used with moderate safety if animals suspected of having exophthalmos were paired after injection with controls of equal body weight   This procedure would be neither quantitative or accurate, since the I C D of animals of the same weight varies appreciably *

Mere measurement of I C D after injection, and comparison with values before injection, does not measure exophthalmos but the balance between exophthalmos and loss of weight   It is likely that thyroidectomy has been said to favour the development of exophthalmos simply because it decreases the loss of weight following pituitary administration

Experimental material   All animals have been of the Hampstead in-bred stock   Both sexes have been used, no differences in ocular phenomena having been observed in males and females   A diet of cabbage and hay was given with either bran, oats and middlings, or bran and beet meal Animals were kept in cages of four, in a room maintained at constant temperature

The following extracts were given, by subcutaneous injection —

1   Initial data were obtained using acetone dried pig anter or pituitary, extracted by Wallen Lawrence and Van Dyke s method (11) as modified slightly by Young   The dry yield, representing about 7% of the original powder, was dissolved in normal saline   10 mg to 1 c c Seitz filtered and injected subcutaneously

2   Most subsequent data were obtained using pig s whole pituitary extracted by the same method giving a 12% yield   The solution in saline had a thyrotropic activity of 6 units per c c., and is the pituitary extract used in this work unless otherwise stated

Certain additional data were obtained using —

3   An extract by Van Dyke s method of acetone dried horse whole pituitary

4   An alkaline extract of ox anterior pituitary gland.

Results

Continuous dosage   If growing guinea pigs of about 200 g weight are given daily subcutaneous injections of anterior or whole pituitary extract in suitable dosage, exophthalmos can usually be detected at 24 hours from the first injection , and, in 60 animals so injected, has always been present at 48 hours, except in 2 which were losing weight rapidly   The amount of exophthalmos increases rapidly for 1 to 3 days, and then slowly until about the seventh day   After this it decreases gradually although daily injections are continued, so that at 14 days it is only about 70% of its maximal

* In the strain used, the I C D rises from 24 70 mm. (standard deviation 0 45 mm) at 150 grams, to 26 05 mm (S.D 0 43 mm.) at 200 g body weight

value   Fig 3 gives mean values for a group of 4 animals injected daily
with 2 c c of pig's whole pituitary extract   The individual values in this
and other experiments show that the time course of the change is closely
similar in different animals
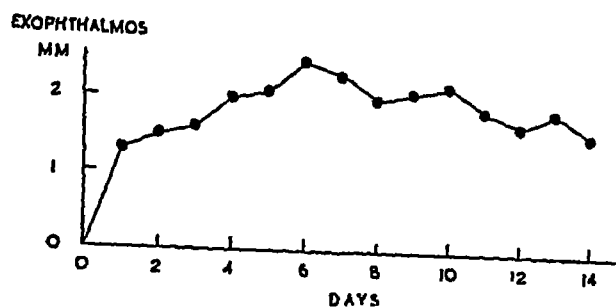
Fig 3   Mean exophthalmos, in millimetres combined protrusion of both eyes, in 4 animals
injected daily with 2 c c pituitary extract, plotted against days from the first injection
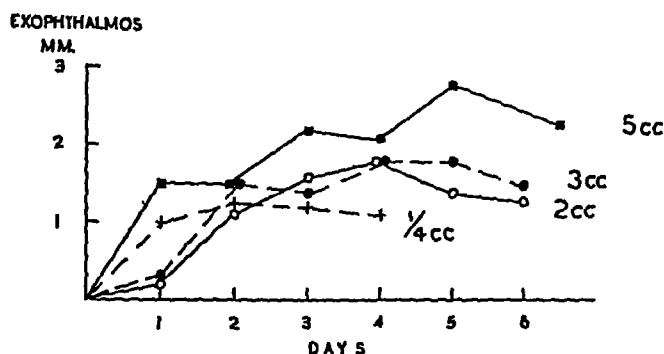
Fig 4   Mean exophthalmos in groups of animals injected daily with different dosage of
pituitary extract

In a single experiment serum was taken from 5 animals in which the
mean value for exophthalmos had decreased from a maximum of 1 8 mm
to 0 5 mm after injection for 18 days   Injections of ½ c c of this serum and
½ c c of pituitary extract were given daily for 5 days to each of four animals
These developed exophthalmos, and enlargement of orbital structures, equal
to that in four control animals injected only with pituitary extract

The amount of exophthalmos attained on daily injections depends
upon the dosage given   Extracts of pig's whole pituitary containing 12
units of thyrotropic hormone cause a mean exophthalmos of about 2 mm ,
and larger dosage causes only a moderately greater increase in protrusion
(Fig 4)   It is shown later that this is probably due to limitation in movement
of the eye, since the retro-orbital swelling that causes the exophthalmos is
further increased by higher dosage   The greatest exophthalmos observed
in animals of about 200 grams has been 3 4 mm , and values exceeding
3 mm are not unusual in individual animals on dosage of 20 or more units
of thyrotropic hormone daily

Discontinuation of dosage If, during the second week of injections, dosage is discontinued, the exophthalmos regresses little or no more rapidly than if injections are continued During the first week, however, interruption of dosage causes rapid regression of exophthalmos which may be halved within 24 hours of the missed dose If dosage is discontinued after 2 injections, exophthalmos has disappeared by the 14th day If a single injection only is given, the eyes have returned to normal by the 5th day and in some cases exophthalmos does not develop

Resumption of dosage Exophthalmos which has partially regressed as a result of a missed dose during the first week is restored to about its former value within 24 hours of resuming dosage Exophthalmos has also been produced when that due to two previous injections has subsided, although the response in each of 4 animals was less than that produced by equal dosage 14 days earlier

Exophthalmos has also been produced in larger and in adult animals but its behaviour has not been investigated quantitatively since a satisfactory base-line of I C D against weight is harder to obtain, and since loss of weight is greater

No differences have been observed in the behaviour of the exophthalmos produced by anterior or whole pituitary extracts, or by extracts of pig, horse or ox pituitary No exophthalmos or changes in weight or composition of orbital structures have resulted from single or daily injections of posterior pituitary extract in dosage high enough to produce considerable antidiuretic action in the animals

No exophthalmos or orbital changes have been obtained with the following control solutions —

1 Normal saline in large doses either subcutaneously or intraperitoneally

2 Pituitary extract boiled for 20 minutes

3 Thymus extract of nitrogen content about equal to that of the pituitary extracts used

4 Thyroxine solutions containing 0 8 mg of thyroxine The orbital findings after thyroxine injection have not been found to differ in any way from those produced by other factors causing an equal loss of body weight, and the changes in I C D are similarly related to the weight loss Factors causing loss of weight have been epidemics of diarrhoea and, in the case of one group of animals, the accidental use of an infected thymus extract

It can therefore be stated that *exophthalmos results from anterior pituitary administration in a regular manner which can be predicted quantitatively* In its early stages it regresses if dosage is discontinued, and in later stages even if it is continued No systematic attempt has been made to discover a system of dosage which causes persistent or progressive exophthalmos In a few experiments, dosage increasing daily

by constant amounts or in constant proportion failed to produce such effects Prolonged administration of small dosage has not been attempted

Effect of thyroidectomy It has been stated that greater exophthalmos is caused in thyroidectomised than in normal guinea pigs by pituitary injections, and theories as to the origin of the severe post-operative exophthalmos in man have been based on this statement In fact, however, thyroidectomy merely has its effect by decreasing the loss of weight on pituitary injections, so that the I C D rises higher in such animals than in unoperated ones The amount of exophthalmos, however, is equal in normal and thyroidectomised animals Fig 5 shows the exophthalmos developed in 4 normal and 4 thyroidectomised animals and, except that one normal only developed transient exophthalmos, the effect is clearly the same in the two groups, The results of two experiments using pig's anterior pituitary extract are given in Table I It is quite clear that, when allowance is made for concomitant weight changes, thyroidectomy has no effect on the amount of exophthalmos resulting from pituitary injections
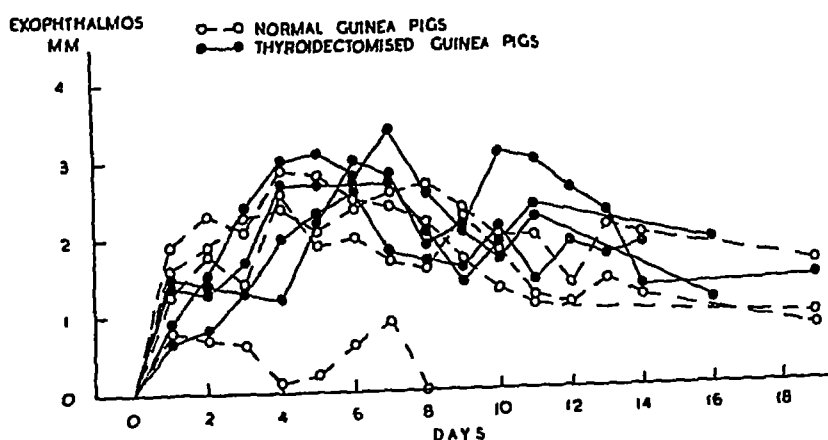
Fig 5 Exophthalmos in 4 normal and 4 thyroidectomised animals injected daily with pituitary extract

TABLE I

Exophthalmos in thyroidectomised and normal animals

Mean exophthalmos (mm)

	Day	1	2	3	4	5	6
Expt I	4 normals	0 1	1 2	1 7	1 8	1 7	1 4
	4 thyroidectomised	0 4	1 1	1 4	1 7	1 6	1 4
Expt II	4 normals	0 2	1 1	0 7	1 6	—	—
	4 thyroidectomised	0 5	0 7	1 6	2 0	—	—

Mechanism of exophthalmos The measurement of inter-corneal distance includes dimensions of skull and eye-balls which do not alter during the

development of exophthalmos It is found, moreover, that the eyes sink in after death by an approximately equal amount in normal and exophthalmic animals Exophthalmos is thus associated with a swelling of the retro-bulbar orbital tissues, the thickness of which may be increased several fold Table II gives mean values in 8 animals injected with pituitary extract compared with data from 8 control animals injected with doses of thyroxine causing an equal loss of weight In each case injections were given daily for four days, and the animals killed on the fifth day It will be seen that a mean exophthalmos of 1 9 mm in life, or 1 5 mm post-mortem, is largely due to enlargement of the retro-bulbar tissues, the thickness of which is doubled The table gives also the difference in weight of these orbital tissues in the two groups, and a figure for the exophthalmos to be expected if the eye is simply displaced forward like a piston by the increase in tissue bulk This figure is obtained by dividing the increase in tissue weight by the measured cross-sectional area of the eye, and agrees reasonably with the exophthalmos observed Calculation from weight changes is adequate since the specific gravities of these tissues is nearly unity *

TABLE II

Dimensions and weights of orbital tissues in exophthalmos

	Pituitary injected animals	Thyroxine injected animals	Difference
Initial weight	232 g	238 g	—
Final weight	189 g	194 g	—
I C.D in life	27 2 mm	25 3 mm	1 9 mm
I C.D post-mortem	25 5 mm	24 0 mm.	1 5 mm.
Width of skull	6 5 mm.	6 6 mm	—
Width of eye balls	16 8 mm	16 3 mm	—
Thickness of orbital tissues	2 2 mm	1 1 mm.	1 1 mm.
Weight of orbital tissues	404 mg	311 mg	93 mg
Cross sectional area of eyes	—	—	76 sq mm
Exophthalmos expected	—	—	1 2 mm.

The thickness of the orbital tissues is that measured along the intercorneal axis It is increased out of proportion to the increase in weight of orbital tissues because the eye is more displaced in exophthalmos than the tissues forming the remainder of the lateral wall of the orbit

Not only is exophthalmos accounted for quantitatively by the amount of orbital swelling produced, but the sinking of the eye associated with wasting is similarly related to the observed tissue changes Fig 6 compares the changes in eye position observed in animals with the changes to be expected from the alteration in bulk of orbital contents Cases of

* The dorsal lachrymal gland containing 24% fat, has a specific gravity of about 1 00 while the ventral lachrymal gland with 5% fat has S G 1 06 Eye muscles have a S G of about 1 04

exophthalmos are in pituitary injected animals, and are related to the excess of orbital tissue over that in normal animals of the same weight   Cases of enophthalmos are in animals that had lost weight either from thyroxine administration or other causes of wasting   Points plotted as discs indicate the variability of orbital contents in normal animals
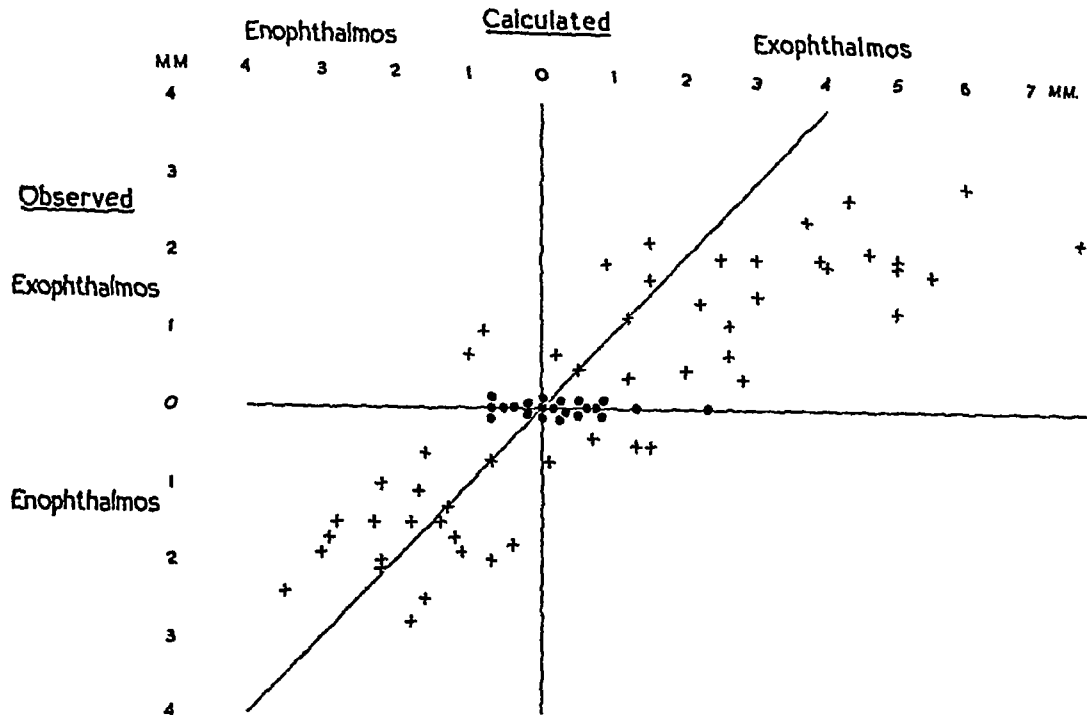
Fig 6   Relation between observed displacements of the eye and those calculated from the changes in bulk of the orbital contents

Crosses, above the horizontal axis —exophthalmos in pituitary injected animals

Crosses, below this axis —enophthalmos in wasted animals

Discs —calculated position of the eye in normal animals

The line corresponds to agreement with prediction

It should be appreciated that the measure of enophthalmos used is the sinking of the eye from its prominence before loss of weight started   In wasted animals, the I C D remains about normal for the body weight, that is, equal to that of a growing animal at the same weight Thyroxine has an effect equal to that in loss of weight from any other cause, and to describe thyroxine as a hormone producing enophthalmos is misleading except in this general sense

It will be seen from Fig 6 that a change in the bulk of orbital tissues produces approximately the expected change in position of the eye, except that considerable orbital swelling does not produce exophthalmos in excess of about 3 mm   This is probably due to an extensive peribulbar protrusion of tissue that occurs in such cases   It is clear that measurements of exophthalmos form an inefficient index of the intensity of the process causing the protrusion, and that a closer analysis may be based on the orbital tissue changes

Swelling of orbital structures The retro-bulbar orbital tissues in the guinea pig consist of the eye muscles, vessels and nerves and the dorsal (or Harderian) lachrymal gland Very little orbital fat is present in the 200 gram animal. The ventral lachrymal gland is partly retro-ocular and partly infra-ocular The dorsal gland represents about half of the orbital tissues by weight In the following account the term " orbital tissues " refers to all orbital structures except the eye-ball , " eye muscles " includes the four recti and two obliques, but not the four small retractores bulbi , and " residual tissues " covers all " orbital structures " except the two lachrymal glands and the " eye muscles," and includes vessels, nerves, retractor muscles, connective tissue, some adipose tissue and the sheath of the dorsal lachrymal gland

The normal weights of orbital structures in growing animals are closely related to body weight as shown in Fig 7 Further, in an animal which has lost weight owing to thyroxine administration or other cause, these structures weigh about the same as in a growing animal of equal body weight (*see* Table III) It is thus possible to determine the increase in weight of orbital structures in animals which have received pituitary extracts and despite loss of weight The normal variability in weight of these structures is such that a 20%
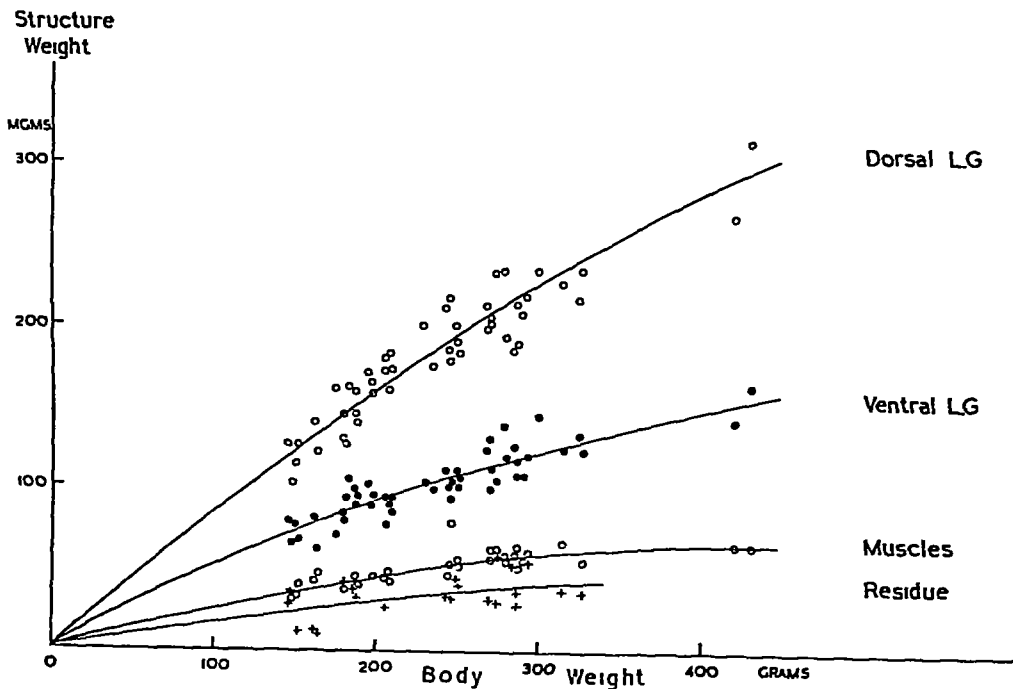
Fig 7 Weight of orbital structures plotted against body weight
L G = lachrymal gland.

increase in the muscles or either lachrymal gland in a single injected animal is significant statistically (to 96% probability), and smaller increases can be detected using groups of animals

TABLE III

Weight of orbital structures in wasted animals

	Mean weight in —		Difference	Standard error of difference
	8 animals on thyroxine	Normal animals		
Body weight (g)				
Initial	238	—	—	—
Final	194	194	—	—
Structure weight (mg)				
Dorsal lachrymal gland	162	155	+ 7	8
Ventral lachrymal gland	78	81	− 3	4½
Muscles	43½	44	− ½	2
Residue	26½	27	− ½	4

It will be seen that none of the differences is significant

On injection of pituitary extracts, the orbital structures are affected unequally The dorsal lachrymal gland undergoes the greatest relative enlargement, of extent depending upon the dosage given The percentage enlargement of the ventral gland is about half — and that of the eye muscles about a quarter — that of the dorsal gland The mean results from 7 experiments, each involving 4 or more animals and causing moderate exophthalmos, were —

dorsal lachrymal gland   32% enlarged
ventral lachrymal gland   15% enlarged
eye muscles   8% enlarged

The sheath of the dorsal gland, normally a thin fascial membrane, swells into a thick gelatinous mass, and may cause doubling of the weight of " residual tissues " It is difficult to avoid removing and weighing fragments of this sheath with the eye muscles, which may account for their apparent enlargement, but it seems probable that true enlargement of the eye muscles occurs Changes in other residual tissues are obscured by the swelling of the sheath, but Soxhlet extraction of the total residual tissues shows that the small amounts of orbital fat in animals of this size are not increased in exophthalmos

Nature of increases The increases observed in orbital structures are predominantly due to a raised water content, with only slight changes in the dry fat-free and fat components of these tissues This is in contrast to the changes in wasting, when the loss of orbital tissue is associated with a decrease in the water percentage of the structures Table IV compares the findings in two groups of 8 animals treated for 4 days with pituitary extract in one case and thyroxine in the other, so that loss of weight and final body

weight were equal in each group   A rise in water percentage occurs in each structure with exophthalmos, and a comparison of the absolute weights of the chemical components of these structures shows that the change involves only the water content   In this experiment the orbital water content was increased by 50% without change in the water-free components

TABLE IV

Changes in water content of orbital tissues in exophthalmos

	Water percentages			Absolute weights (mg)			
				On thyroxine		On pituitary	
	Normal	On thyroxine	On pituitary	Water free	Water	Water free	Water
Dorsal 1 g	64 4	60 3	70 1	64	98	63	149
Ventral 1 g	74 7	72 7	77 0	21	57	19	63
Muscles	82 3	81 7	84 1	8	36	8	43
Residue	80 8	71 6	88 6	8	19	7	51
			Total	101	209	97	307
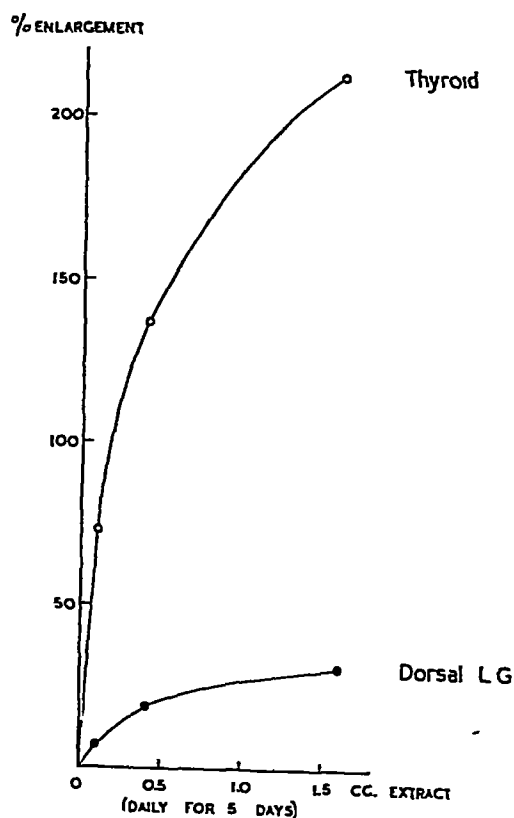
Fig 8   Mean percentage enlargement of thyroids and dorsal lachrymal glands in groups of animals injected daily for 5 days with different doses of pig s whole pituitary extract

The dorsal lachrymal gland may show distension of the acini, but the change is presumably one of œdema in view of the enlargement of the sheath of this gland, which is greater than would be likely to arise as a result of secretory changes in the gland itself. It should be emphasised that this nature of the change has been established only for the 200 gram animal developing exophthalmos rapidly as a result of high dosage with pituitary extract

Method of assay The enlargement of the dorsal lachrymal gland affords a convenient method of assaying the pituitary factor responsible for exophthalmos in 200 gram animals. The percentage increase in its weight is considerably less than that of the thyroid glands. Fig 8 shows that in an experiment with ox whole pituitary, the relative enlargement of this lachrymal gland was about one seventh that of the thyroids for large doses, and rather less on smaller dosage. Daily administration for 5 days of extract containing one unit of thyrotropic hormone (7) caused a 10% increase in the dorsal lachrymal gland * It has not been established that exophthalmos is caused by the thyrotropic hormone itself. In preliminary experiments, however, with pig, horse and ox pituitary, which have widely different thyrotropic activities, the enlargement of the lachrymal gland has varied in approximate proportion to the thyroid enlargement

Changes in other body tissues Smelser observed œdema of orbital fat in adult guinea pigs, and regarded this as the local expression of a generalised œdema of body fat depots (10) The raised water percentages which he observed in fat depots are due, however, not to an increase in their water content, but to a decrease in their fat content, which is a normal accompaniment of wasting This has been established in growing animals by a quantitative study of tissue fat, and in particular of two well-demarcated depots lying in the axilla and on the psoas muscle within the abdomen The absolute weight and average water percentage of these depots were first determined in normal animals The corresponding values were then investigated in animals that had lost weight, either from pituitary injection, or from thyroxine or other causes It was found that the water percentages in these and other depots are raised in wasted animals, and that the elevation varies with the degree of body wasting In axillary fat the percentage rises from a normal value of 35% to about 65% in an animal which has lost 30% of its body weight, while the value in abdominal fat rises from 26% to 70% Equal values are observed in animals injected with pituitary extracts and in those which have lost an equal amount of weight from other causes

Axillary Fat   Abdominal Fat

Fig 9   Water percentage in two fat depots, plotted against estimated loss of weight of the depot in wasted animals.
        The lines represent the values expected on the assumption that loss in depot weight is due to loss of water free material without change in the absolute water content.

Moreover, if the water percentage is plotted against the estimated percentage loss of weight of the fat depot itself, the values correspond reasonably with those which would be expected if the loss of weight had been due to loss of fat only without loss of water from the depot (Fig 9) It will be seen that this applies equally to animals treated with pituitary extract and to controls   It is thus clear that the orbital gain in water is not explicable by the raised water percentage in body fat depots

Discussion

It is clear from the present work that the exophthalmos which results from pituitary injections in the 200 gram guinea pig can be accurately assessed, and is then found to be reproducible and quantitatively related to the amount and duration of dosage   The course of the exophthalmos has been uniform in individual animals, irregularity being usually associated with rapid loss of weight in an animal injected when presenting too great an initial body weight   The regression of exophthalmos after about a week of injections has been equally uniform   It is of interest that this occurs at about the period when anti-hormones might be expected to develop, although a single experiment failed to demonstrate any circulating substance capable of inhibiting the development of exophthalmos in other animals   If this indicates a local resistance, it is possible that certain orbital structures may become resistant early, and that other structures may cause exophthalmos

later in a course of injections   It is desirable that the presence of exophthalmos at later stages of pituitary administration should be investigated by quantitative methods which allow for concomitant changes in body weight The nature of any orbital changes produced in such cases or in adult animals should be compared with those produced rapidly in growing animals

The exophthalmos studied is largely due to an increase of water in the orbit, which is probably to be regarded as a generalised orbital œdema This contrasts with exophthalmos in human Graves' disease which is due largely to an increase in orbital fat (8) *   At the same time it seems uncertain that, even if a common factor were responsible for both types of exophthalmos, it would produce similar results when effective at such widely different rates   Exophthalmos in the guinea pig has been largely established in 48 hours, while a moderate protrusion of 4 mm in man may develop in a year

It is important to emphasise that thyroidectomy has *no* effect, at least in 200 gram animals, on the amount of exophthalmos produced by pituitary injections   The absolute increase in I C D is often greater, corresponding to the smaller loss of weight in operated animals   Thus one experiment gave mean exophthalmos of 1 3 and 1 4 mm in thyroidectomised and normal groups   In the former the I C D had risen 1 7 mm with a rise of 16 grams in body weight at the sixth day   In the latter group, the I C D had risen 1 2 mm despite a 2 gram fall in weight   It seems probable that similar effects may have caused an incorrect interpretation of the influence of thyroidectomy in previous work, particularly where naked eye estimates of exophthalmos were made   An extensive hypothesis has been built on the supposed influence of thyroidectomy, postulating that removal of the thyroid calls forth excess of thyrotropic hormone from the pituitary owing to absence of circulating thyroxine which previously inhibited its output   An excess of this hormone was held *to be responsible* for progressive or " malignant " exophthalmos which may occur in man after thyroidectomy or in association with a low basal metabolic rate   The present work demonstrates that this hypothesis may be based on a misinterpretation of the effects of thyroidectomy on the development of exophthalmos in guinea pigs

SUMMARY

1   When growing guinea pigs are given anterior pituitary extract in suitable doses by subcutaneous injection, exophthalmos constantly results within 48 hours

2   The amount of exophthalmos depends upon dosage, moderate exophthalmos resulting from daily injection of extract containing between 5 and 10 units of thyrotropic hormone

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* The fat content of the eye muscles was not systematically examined in the present work, which was complete before that on man was started

3 The exophthalmos increases for a week, and then decreases despite continued injections

4 Exophthalmos is equal in normal and thyroidectomised animals

5 The exophthalmos is quantitatively accounted for by œdema of the orbital tissues, particularly affecting the dorsal lachrymal gland and its sheath

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NOTE —Valuable papers by Smelser which have appeared since completion of the present investigation (*Amer J Anat* 1943 72, 149 and its references) have dealt with the structures causing exophthalmos and the effect upon them of sympathectomy thyroxine and enucleation of the globe Allowance has not however been made for the effects of changes in body weight upon the I C.D or on the weight of orbital structures Aird (*Arch Ophthal N Y* , 1940 24, 1167) has presented evidence that thyrotropic hormone is responsible for the orbital changes

MASSIVE HEPATIC NECROSIS AND DIFFUSE HEPATIC FIBROSIS

(ACUTE YELLOW ATROPHY AND PORTAL CIRRHOSIS)   THEIR
PRODUCTION BY MEANS OF DIET

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THE present interest in the production of hepatic injury by means of diet dates from a paper by Rich and Hamilton (27) in which the claim was made to have produced cirrhosis of the liver in rabbits in this way   Almost simultaneously similar claims were made by other authors (5, 7, 15, 29, 33) and, adequate confirmation being forthcoming, it is now securely established that hepatic injury can be produced by dietetic means   But as yet there is no agreement as to what these means are   At one time or another practically every known dietary constituent, save carbohydrate, has been indicted as the responsible factor and the uncertainty in this field is so great that in a recent review Bollman (6) wrote that dietary injury to the liver could apparently be brought about by many different ways   In our opinion this uncertainty is in part accounted for by failure to distinguish between the different types of lesion produced   With increasing frequency it has been reported that a small and inconstant proportion of the animals receiving the diet designed to produce cirrhosis of the liver showed hæmorrhages or necrotic lesions in the liver (12, 15, 16, 33) but, as far as we have been able to discover, only one group of investigators has considered that these lesions might represent a different condition from the early lesions of cirrhosis   On the basis of observations that addition to the diet of different amino-acids influences to a different extent the incidence of necrotic or of cirrhotic lesions in the experimental animals Daft and his colleagues (11) have suggested that the two lesions might be distinct   The majority of workers, however, assume that the hæmorrhages or necrotic lesions are a stage in the sequence of events leading to cirrhosis of the liver, and the reason for this assumption is to be found in the fact that so far it has not been possible to produce the necrotic lesions separately from those of " cirrhosis "   The work presented in this paper shows that these necrotic lesions are distinct from portal cirrhosis not only in appearance, clinical manifestations, and sequelæ but also in causation

The present work arose out of our investigations into poisoning by trinitrotoluene which showed that the susceptibility of rats to this poison was determined by the composition of the diet so that animals taking a relatively low protein, high fat diet developed typical necrosis of the liver (19) Pure food stuffs were not at that time available but later they were provided It was then found that when pure corn starch was substituted for bread, and lard for bacon fat, in our diets typical hepatic necrosis developed in animals who were not receiving T N T The most obvious difference between the crude and the pure diets was that the latter were poorer in protein because of the removal of the "Wheatmeal" flour (National flour) which contained at that time 12 5% of protein We therefore constructed a series of diets containing different proportions of casein and found that when the casein intake fell below a certain level necrosis developed These diets did not lead to the development of a true portal cirrhosis The fibrosis of the liver which they produced was the entirely different condition of nodular hyperplasia which clearly began as scarring at the site of previous necrosis Other diets did, however, produce a diffuse hepatic fibrosis which had all the features of portal cirrhosis These latter diets had in common the single property that they rapidly produced gross fatty infiltration of the liver It is thus possible to distinguish two distinct types of hepatic injury due to diet The first is due to lack of protein and appears to be a deficiency disease arising, probably, as the result of a deficient supply of an amino acid The second is due to the effect of long continued fatty infiltration of the liver whether this is produced by an excess of fat or a deficiency of lipotropic factors in the diet The injury due to protein deficiency is characterised by the development of massive hepatic necrosis which leads, in the survivors, to post-necrotic scarring and nodular hyperplasia The injury due to fatty infiltration of the liver is characterised by the gradual development of a diffuse hepatic fibrosis which progresses to a condition indistinguishable from portal cirrhosis In discussing our results we shall first describe the clinical and pathological features of the two lesions and then the factors influencing the development of each

METHODS

Animals White "Wistar" rats,* ranging in weight from 120 g to 150 g were used All the animals in any experimental or control group were of the same sex and of approximately the same weight The rats of each group were kept in a single cage, unless the number exceeded 10, in which case more cages were used Twice a week each group of animals was weighed and the average weight of the rats thus determined

Histological examinations The animals were killed by stunning Samples were fixed in 10% formol saline Liver samples were always taken from the middle of the large left lobe which overlaps the stomach and, if doubtful lesions were seen in other parts of the organs, samples were taken from these places also Paraffin sections were stained as a routine with hæmatoxylin and eosin When necessary other paraffin sections were stained for reticulin fibres by Laidlaw's method (20) and for fibrous tissue by van Giesen's stain and iron hæmatoxylin Frozen sections were stained for fat with Scharlach R

* These were obtained from the breeding colonies of Glaxo Laboratories and of Messrs Boots

Estimation of liver fat This was carried out by a modification of the method described by Leathes and Raper (21)

* *Feeding* Eight grammes of food, irrespective of the nature of the diet was allowed to each animal daily The animals were fed at the same time each day At the end of twenty four hours the residue of food was collected and weighed. In calculating the amount of food eaten in the case of diets which required moistening, allowance was made for the proportion of water in the residue Water was not restricted

Diets

A *Ingredients*

1 *Salt mixture* All diets contained 3% of salt mixture

2 *Vitamins* All diets contained 1% of cod liver oil All animals received daily supplements of water soluble vitamins to the following amounts thiamin hydrochloride 20 μg, riboflavine 25 μg pyridoxin 20 μg †calcium pantothenate 100 μg All vitamins were added to the food, in the case of fatty diets when these were made up in bulk at approximate intervals of a week in the case of carbohydrate diets immediately before the food was dispensed to the animal

3 *Choline* In the experiments in which choline was used it was added to the food in amounts of 4 mg /rat/day that is 50 mg per 100 g of food

4 *Yeast* ‡The yeast powder was prepared from a pure strain of yeast grown under constant conditions The powder was produced by drying the whole yeast at 30°C after squeezing through perforated plates The dried powder contained 44% of protein the remainder was almost entirely carbohydrate Professor J H Gaddum of the University of Edinburgh kindly estimated the choline content of the powder and found that it contained 13 μg of choline per gramme of powder

5 *Casein* Glaxo Laboratories Grade A casein was used

6 *Fat* ** Lard was used for the fatty diets 5% of arachis oil was added to all the carbohydrate diets

7 *Carbohydrate* ** The best quality crushed maize starch was used.

B *Composition of the diets* Two groups of diets were used and distinguished by the name of the predominating ingredient as a Fat Diet ' or a ' Carbohydrate Diet " respectively The individual diets in each group can be further distinguished from each other by the quantity and kind of protein included.

1 *Fat diets* All fat diets contained 50% of lard, 1% of cod liver oil 3% of salt mixture and the vitamin supplements The remainder of the diet was made up of carbohydrate and protein Thus an 8% casein, fat diet contained 50% of lard 3% salt mixture, 1% cod liver oil, vitamins 38$\%$, maize starch and 8% casein A 7 2$\%$ yeast protein fat diet contained the same amounts of fat salt mixture and vitamins but 7 2% yeast protein, 8 8% yeast carbohydrate and 30$\%$ maize starch

2 *Carbohydrate diets* All carbohydrate diets contained 3$\%$ salt mixture, 1$\%$ cod liver oil 5$\%$ arachis oil and the vitamin supplements The remainder of the diet was composed of carbohydrate and protein Thus a 16$\%$ casein, carbohydrate diet contained 3$\%$ salt mixture, 1$\%$ cod liver oil 5$\%$ arachis oil vitamins, 75$\%$ maize starch and 16$\%$ casein. A 4$\%$ casein 3 6$\%$ yeast protein, carbohydrate diet contained the same amount of arachis oil, salt mixture and vitamins but 3 6% yeast protein, 4 4$\%$ yeast carbohydrate 4$\%$ casein and 79% maize starch

* We are indebted to Dr Charlotte Himsworth for the feeding of the animals

† We are indebted to the Glaxo Laboratories for the gift of this substance

‡ We are indebted to the Distillers Company Ltd for the gift of supplies of dried yeast powder

** We are indebted to Professor Sir J C Drummond of the Ministry of Food for obtaining permission for us to purchase supplies of these rationed commodities

I   COMPARISON OF MASSIVE HEPATIC NECROSIS AND DIFFUSE HEPATIC
                          FIBROSIS

CLINICAL

The clinical features of the development and course of massive hepatic necrosis and of diffuse hepatic fibrosis show striking differences   Hepatic necrosis is a devastating illness which appears suddenly in a hitherto healthy animal , diffuse hepatic fibrosis is a gradual process which slow and quietly progresses over a long period

Massive hepatic necrosis and its sequelae

After the animal has been put on the protein deficient diet there is invariably a latent period before hepatic necrosis develops   The delay depends upon the nature of the diet , some diets produce necrosis quickly, others slowly   The shortest delay observed has been 28 days, but usually, even with the most effective diet, it is between 40 and 60 days   With other diets the delay may be 100 days or more   During this period the animal does not seem ill   Appetite is unimpaired and activity normal and, unless the protein in the diet is grossly insufficient, the animal gains weight   The liver during this period shows no abnormality   Then suddenly the animal ceases to eat and huddles in the corner of the cage   In severe cases death, often preceded by convulsions, follows in a matter of hours   In animals which have been severely affected but which have not been killed outright, there follows a period of two or three weeks of obvious illness   During this period the animal eats little, its weight falls and jaundice may develop If it survives this period it becomes more active, but it does not regain the weight it has lost, and usually continues to lose weight slowly for some weeks   Examination of the rat at this period shows an animal with sparse fur, general emaciation, and often an abdomen distended with fluid   At autopsy subcutaneous oedema, peritoneal, pleural and pericardial effusions and a deeply scarred and distorted liver are found   In less severe cases after a week or two of illness the animal recovers only to relapse some weeks later   In others there is a mild and transient illness followed by complete clinical recovery and, if it were not for the evidence at autopsy, it would be doubtful whether the animals had ever been affected   In all cases the severity of the illness and of its sequelae are found to be related to the amount of liver damage

Diffuse hepatic fibrosis

The development of diffuse hepatic fibrosis is a gradual process which, uninterrupted by sudden illness, leads steadily but slowly to deterioration of health   Throughout the experimental period the animals remain active, their appetite is good and the only indication of the course of events is given

by their change in weight   During the first 100 to 150 days of the experiment their weight curve rises to a plateau   Then for some weeks it declines slowly The curve now becomes erratic , rapid falls in weight alternate with equally rapid recoveries, but the general trend is still downwards   After some weeks of such variations in weight the animal usually dies of some inter-current illness, such as pleuropneumonia, and diffuse fibrosis is found in the liver   At any time after the weight curve becomes erratic microscopic evidence of the lesion may be found at autopsy but, even with our most effective diet, macroscopic evidence is rarely present before the 200th to 250th day

PATHOLOGICAL

Massive hepatic necrosis

We distinguish generalised or extensive massive necrosis of the liver from partial massive necrosis of the liver (14)   In the former every lobe of the organ is extensively involved (Fig 1) , in the latter only the left lobes (Fig 2)

Considering generalised hepatic necrosis first it is found that, in animals which are killed within a few hours of becoming severely ill, the whole liver is swollen, maroon in colour, covered with a mesh of dilated vessels and sparsely spotted with petechiæ (Fig 1)   At this stage macroscopic examina-tion shows large tracts of liver tissue in which all the parenchymal cells are dead, but still in situ, alternating with areas in which varying numbers of liver cells survive   Livers from severely ill animals which have survived a day or two show a different appearance   Whilst they are still swollen and show a mesh of dilated vessels they are uniformly yellow in colour and closely resemble both macroscopically and microscopically, human livers with " acute yellow atrophy "   Should the animal survive a week or two the picture changes again   The liver is flabby and no longer appears swollen Its general colour is a dull yellow but on its surface are sharply demarcated and slightly sunken brick red areas   Its resemblance to the " subacute red atrophy " of human pathology is apparent   Very occasionally animals with such extensive necrosis survive for several weeks longer to develop œdema and effusions   In such cases the liver is found markedly distorted   Its surface is cut into by deep and irregular scars (Fig 3) and the intervening substance is covered with projecting nodules of liver tissue   A similar appearance of the liver has long been recognised in human pathology as the sequel to " acute atrophy " and in this country has usually been distinguished from the diffuse hepatic fibrosis of portal or Lænnec's cirrhosis by the term nodular hyperplasia

It appears probable that this type of necrotic lesion was first produced by Weichselbaum (35)   In 1935 he described the production of " hæmorr-hages " into the liver by dietetic means   It seems apparent from the nature

of the diet he used that he must have been producing the acute necrosis we have described, although he did not recognise the lesions as such Since then several groups of workers (12, 15, 16, 33) in attempting to produce " cirrhosis of the liver " have produced also hepatic necroses which appear identical with ours But this lesion " could not be produced at will and was not a regular occurrence " (15), and has been in general regarded as merely a transient stage in the sequence to cirrhosis

Partial hepatic necrosis differs from generalised hepatic necrosis only in the distribution of the lesions By killing animals at appropriate times specimens showing necrotic processes in the early " acute yellow atrophy " and " subacute atrophy " stages can be obtained But in all cases the necrotic processes are localised to particular parts of the liver The large left lobe of the liver overlying the stomach is always involved (Fig 2) Usually the omental and retro-gastric lobes are also affected In more severe cases the central lobe is affected up to and slightly beyond the insertion of the falciform ligament Occasionally the summit of the right lobe which rests in the cupola of the diaphragm is also involved The main mass of the right lobe and middle lobe appear perfectly normal In animals killed some weeks after the onset of the illness the areas picked out in partial hepatic necrosis are shrivelled and scarred (Fig 3) whilst the rest of the liver looks larger than usual—an impression which is confirmed by finding that the weight of the whole organ is approximately normal

This localisation of the necrotic lesions in partial necrosis to particular parts of the liver has been noted in human cases (21, 32) The problem is to explain why in a particular liver, or even in one and the same lobe of that liver, areas in which every parenchymal cell is dead alternate with areas in which every liver cell appears perfectly normal Superficially the areas of necrosis resemble infarcts but careful search has failed to reveal any blockage of the hepatic vessels It is not unusual to find the area of necrosis accurately confined to that part of the left or middle lobes which is pressed upon either by a neighbouring lobe, an adjoining viscus or the costal margin But pressure explains only a minority of the cases The explanation is suggested by those cases in which the necrotic process involves the middle lobe up to and beyond the insertion of the falciform ligament This part of the middle lobe is known in man and some other animals to receive its blood supply from the left branch of the portal vein It is further known that in the portal vein the blood coming from the superior mesenteric vein tends to the right, and so passes mainly up the right branch of the portal vein to the right lobes of the liver, whilst the blood from the splenic vein keeps mainly to the left, and so reaches the left lobes of the liver (9, 12, 19) Thus the right lobes of the liver are supplied with blood which comes predominantly from the small intestine , the left lobes by blood from the spleen and large intestine To test whether a similar distribution of the portal blood occurred in rats we anaesthetised some normal rats and injected Indian ink into their spleens Within a few seconds the Indian ink had

been carried to those parts of the liver which are affected in partial hepatic necrosis

It thus appears that in partial massive necrosis of the liver lesions occur in those parts of the organ which receive blood from the spleen and large intestine whilst they do not occur in those parts of the liver which receive blood from the small intestine   This implies either that a noxious substance is conveyed to the liver by the blood from the spleen and large intestine, or that blood from the small intestine contains a substance which prevents the liver cells developing necrosis   But we have also evidence that pressure facilitates the production of necrosis and, as pressure must curtail the blood supply to the part pressed upon, then, if the substance carried by the blood were injurious, pressure should protect the part against the development of necrosis   It appears, therefore, that a substance is present in the blood from the small intestine which prevents liver cells going into the state of necrosis   The survival of the right lobes of the liver in cases of partial hepatic necrosis would be adequately explained on this hypothesis

Diffuse hepatic fibrosis

It is not until an animal has been taking the appropriate diet for a long period that the macroscopic changes of diffuse hepatic fibrosis become evident   Then the liver shows a finely granular surface and its colour is buff by reason of the considerable amount of fat it contains (Fig 4)   Long before this macroscopic evidence appears, however, microscopic sections stained for reticulin fibres reveal that stretching between each portal tract are fine fibres which circumscribe the enclosed lobule (Fig 6)   This lesion is uniform throughout the liver   By examining the livers from animals killed at suitable intervals it can be seen that, as the condition progresses, more and more reticulin fibres are laid down around the lobules until these are separated by relatively thick bands of maturing fibrous tissue   By the time these changes are established changes are occurring within the lobules themselves , some increase in size, some are subdivided by invading fibrous tissue   Finally the typical picture of portal cirrhosis is obtained (Fig 7)   At no time in the course of the development of the lesion is necrosis evident , at all times the liver cells are heavily laden with fat

Although numerous claims have been made to have produced " cirrhosis of the liver " in rats (4, 5, 10 11, 12, 17, 29) the acceptance of these must be modified by two considerations   First no investigator seems to have differentiated between " post-necrotic scarring " and " diffuse hepatic fibrosis " , second the diets used by most workers in this field have been such as to produce both lesions   As a result the following statement has received general acceptance , " Rats with dietary hepatic injury exhibit, in sequence, changes that vary from diffuse necrosis resembling human acute or subacute yellow atrophy to advanced portal cirrhosis " (17)   Whilst we are convinced that a true diffuse hepatic fibrosis has been produced in rats, we suspect,

however, that the majority of the lesions which have been described as such are really examples of post-necrotic scarring

It is apparent that diffuse hepatic fibrosis differs from post-necrotic scarring of the liver, not only as regards its clinical history and speed of development, but also in pathological appearances   First, the liver in diffuse fibrosis is finely granular (Fig 4) , the scarred liver resulting from necrosis is cut up by deep scars and disfigured by projecting nodules (Fig 3) Second, microscopic examination reveals that in diffuse fibrosis the fibrotic process uniformly affects every lobule throughout the liver (Fig 6 and 7) , after acute necrosis the fibrosis is irregularly distributed so that thick bands of fibrous tissue separate areas in which the liver lobules are normal (Fig 5) Third, necrosis is absent in diffuse fibrosis , in post-necrotic scarring dead and dying groups of cells, often caught up in the fibrous tissue, are prominent features   And fourth, we have never seen diffuse fibrosis without fatty infiltration of the liver cells , hepatic necrosis can, and usually does, occur without there being any excess of fat in the liver

II   THE PRODUCTION OF MASSIVE HEPATIC NECROSIS

Exclusion of an infective factor

Careful post-mortem examinations have been made of all rats dying or killed with this condition and in the vast majority of cases no macroscopic or microscopic evidence of any infection has been found   Samples of affected livers, removed with aseptic technique have been examined for the presence of bacteria and viruses   Professor S J Cowell and Professor A A Miles inform us that cultures of such samples, both under aerobic and anaerobic conditions, showed no growth of bacteria and Dr F O McCallum of the Wellcome Institute for Medical Research failed to show any viruses in similar samples   It thus appears that the hepatic necrosis of our experiments is not associated with any known organisms

To test the possibility that the condition might be due to an infective agent which had not as yet been grown in culture the following experiment was carried out   A colony of rats was purchased from an agency with which we had not previously dealt and taken to an institution where work on this type of necrosis of the liver had never previously been carried out   Half the colony were removed, housed in new cages, given diets which had previously produced necrosis and placed in a room by themselves   The other half of the colony was also given the necrosis producing diets and this group was put in cages which had previously been occupied by rats which had died of acute necrosis   This second group was also housed in a separate room but to this room were brought rats who were suffering from acute necrosis   The food, utensils, etc , in the two rooms were strictly segregated The incidence of hepatic necrosis was exactly the same in the two groups, not only as regards the numbers affected and the time of development of illness, but also in the proportion of animals affected on the different diets

We consider that, taken together, these data justify us in considering that the hepatic necrosis under investigation is not primarily due to an infective agent

The dietetic factor concerned

At the beginning of this paper it was stated that at one time or other practically every known dietary constituent, save carbohydrate, had been indicted as the cause of dietetic injury of the liver   The following conclusions exclude all save one

On certain diets no necrosis occurred showing that, if this lesion could be caused by a vitamin deficiency, the quantity and type of the vitamins supplied to our animals were adequate to prevent its development   On other diets necrosis consistently developed, despite the fact that the animals were receiving the same vitamin supplements   It is thus apparent that the hepatic necrosis seen in our experiments could not be due to a deficient supply of one or more vitamins   Similar considerations exclude a deficiency of any mineral factor as the causative agent   Massive necrosis of the liver can be produced with equal facility by means of diets predominantly composed of fat or predominantly composed of carbohydrate (Tables 1, 2, 3 and 4) provided that the rest of the diet is appropriate   The condition cannot, therefore, be due to variations in the supply of either of these foodstuffs   The remaining dietary constituent is protein and it will now be shown that the development of hepatic necrosis is dependent on the quantity and quality of the protein supplied

Quantity of the dietary protein

It has been customary in the work on dietetic injury to pay attention only to the percentage composition of the diet and to allow the animals an unlimited supply of food   Our experiments show, however, that it is the amount and not the proportion of protein in the diet that matters   We therefore set out to determine systematically at what levels of protein intake massive hepatic necrosis developed when the protein used was, from a biological point of view, of first class value   For this purpose we chose casein as the test protein

Table I shows the relationship between the development of hepatic necrosis and the casein content of the diet both when the diet consists predominantly of fat and when it consists predominantly of carbohydrate It will be seen that necrosis only occurs within a certain range of values for dietary casein and that with values either above or below this range no lesion occurs   The importance of considering the absolute amount, rather than the proportion, of a particular food eaten, is evident from these results   If attention is paid only to the proportion of casein eaten it would appear that 8% of casein in the diet is sufficient to protect again necrosis when the diet is composed almost entirely of carbohydrate whilst this proportion is

TABLE I

Showing that the incidence, severity and rate of development of massive hepatic necrosis is related to the amount of protein (casein) in the diet

Casein %	FAT DIETS				CARBOHYDRATE DIETS				
	16%	8%	0%	1%	16%	8%	6%	4%	2%
Food consumption per rat per diem (average over 11th 40th day)									
(a) Food intake in grammes	6 35	6 30	4 44	4 40	8 0	7 85	7 64	7 18	7 6
(b) Energy value in calories	41 1	40 9	28 8	28 1	34 4	33 7	32 9	30 0	32 6
(c) Calories per 100 g body weight	29 1	33 9	28 1	20 0	24 0	27 1	28 4	27 7	23 6
(d) Protein intake in grammes	1 012	0 503	0 267	0 199	1 280	0 627	0 458	0 288	0 152
(e) Protein per 100 g body weight	0 717	0 418	0 260	0 205	0 844	0 490	0 393	0 258	0 108
Number of animals on diet	9	33	5*	5*	9	16	7*	7*	8
Necrosis of liver —									
(a) Total number and percentage	0(0%)	3(9%)	3(60%)	0(0%)	0(0%)	1(6%)	3(43%)	4(57%)	2(25%)
i Extensive necrosis	0(0%)	1(3%)	3(60%)	0(0%)	0(0%)	0(0%)	1(14%)	3(43%)	1(12 5%)
ii Partial necrosis	0(0%)	2(6%)	0(0%)	0(0%)	0(0%)	1(6%)	2(29%)	1(14%)	1(12 5%)
(b) Time of death, in days, of animals dying with extensive necrosis —									
i Average	—	110	45	—	—	—	73	64	76
ii Range	—	—	39 48	—	—	—	—	38 87	—

The food consumption was estimated over the period 11th to 40th day   Before the 10th day the animals were not certainly adjusted to the diet, after the 10th day many of the animals on particular diets were ill from necrosis of the liver   The time of death is only given for animals dying with extensive necrosis, because animals with partial necrosis rarely die   The proportion of animals dying from necrosis in the affected groups would be larger if from the total number in the group, were subtracted those who were killed before the 30th day in order to see if any changes in the liver were present

* Data regarding further animals on these diets will be found in Table 4

insufficient when the diet consists predominantly of fat Reference to the actual quantities of protein eaten by the animals on the two diets shows however that, owing to their greater appetite, animals receiving the carbohydrate diet consumed 20% more casein daily than did the animals receiving the fat diet The important factor therefore is not the proportion but the amount of casein eaten daily and according to the figures in Table I acute hepatic necrosis occurs when the intake lies between 500 mg and 200 mg per rat per diem

This critical range of casein intake is only approximate and we suspect that it may be narrower than our figures indicate In our experiments the animals were fed in groups The result was that the smaller rats were jostled away from the feeding bowl by the bigger animals and consequently may have obtained less food When the amount of casein supplied fell in the middle of the range this would be of little moment, but at the upper limit of the range there was always the possibility, that in this way, the casein consumption of the weaker animals might be curtailed to such an extent as to cause the development of hepatic necrosis It is possible that in this way the death from hepatic necrosis of three out of the thirty-three animals on the 8% casein, fat diet, and one out of the sixteen animals on the 8% casein, carbohydrate diet might be explained

The most surprising finding in these particular results is that when the casein intake falls below a certain minimum necrosis of the liver fails to develop in animals taking a fat diet and its incidence decreased in the case of those taking a carbohydrate diet This occurs even though the animals survive beyond the time required to develop necrosis Thus, whilst 60% of the animals on a fat diet died of necrosis when the daily intake of casein was 270 mg none died of this lesion when the daily intake was 200 mg , and whilst 43% of the animals on a carbohydrate diet died of necrosis when the casein intake was 290 mg , only 12 5% died from this lesion when the intake was 150 mg (Table I) It is easy enough to accept the observation that animals can be protected against the development of massive necrosis when more than a certain daily minimum of casein is supplied , but clearly a special explanation is required for the finding that a reduction of the casein intake below a certain level also protects

The case of animals on a 4% casein, fat diet can first be considered The most obvious explanation of their failure to develop necrosis is that their food requirements had fallen through inanition That this explanation is not valid can be seen from Table I which shows that these animals utilised as much food as did those on a 6% casein, fat diet, of whom 60% died of massive necrosis The following explanation is suggested. Animals on a fat diet with a daily intake of less than 200 mg of casein waste greatly so that at autopsy the abdominal muscles appear only as filmy brown strands between the peritoneum and skin It is suggested that an intake of casein of less than 200 mg a day is so insufficient for the needs of the animal that body protein is mobilised and that, in the course of this mobilisation, a

TABLE II

Showing that yeast protein is less effective than casein in protecting against the development of massive hepatic necrosis

	FAT DIETS		CARBO HYDRATE DIETS		FAT DIETS	CARBO HYDRATE DIETS
Yeast protein %	14 4%	7 2%	7 2%	Yeast protein % Casein %	3 0% 4%	3 0% 4%
Food consumption per rat per diem (average over 11th to 40th day)						
(a) Food intake in grammes	7 5	6 45	7 4		7 0	8
(b) Energy value in calories	48 8	41 8	31 8		45 5	34 4
(c) Calories per 100 g body weight	38 4	40 0	20 2		40 8	20 2
(d) Protein intake in grammes	1 084	0 401	0 531	Protein intake in grammes	0 532	0 008
				Casein intake in grammes	0 282	0 320
(e) Protein per 100 g body weight	0 852	0 444	0 480	Protein per 100 g body weight	0 474	0 515
				Casein per 100 g body weight	0 249	0 320
Number of animals on diet	16	21	8		24	14
Necrosis of liver :—						
(a) Total number and percentage	9(56%)	15(71%)	7(88%)		13(54%)	3(21%)
i Extensive necrosis	6(38%)	9(42%)	7(88%)		6(25%)	2(14%)
ii Partial necrosis	3(18%)	6(29%)	0(0%)		7(20%)	1(7%)
(l) Time of death, in days, of animals dying with extensive necrosis						
i Average	100	90	44		75	83
ii Range	83 118	45 81	34 52		61 89	70 90

The explanatory footnote to Table I applies equally to this table   It should be noted that, after the first death from massive hepatic necrosis in the group of animals receiving the carbohydrate diet containing 7 2% yeast protein, every subsequent death was due to acute necrosis

protein product which protects against necrosis is set free and used by the liver This suggestion would also explain why starving animals do not develop necrosis The finding that, in the case of animals on a carbohydrate diet, reduction in the protein intake from 290 mg daily to 150 mg daily reduced the mortality rate from necrosis from 43% to 12 5% can be explained on the same lines if we take into account the protein sparing action of carbohydrate (25) At autopsy animals on a 2% casein, carbohydrate diet are not emaciated It is suggested that on this diet the deficiency of protein is such as to cause some degree of mobilisation of body protein but not sufficiently severe to bring about mobilisation of such a degree as would result in the liver receiving adequate supplies of the protective factor

Quality of the dietary protein

The preceding results show that, when casein is the sole source of dietary protein, hepatic necrosis does not develop if the amount eaten daily by the animal is more than 500 mg With other proteins this limit does not apply Reference to Table II will show that when yeast protein is substituted for casein in the diet necrosis occurs at higher levels of protein intake Thus on the diets with a fat basis a daily intake of 1 gramme of casein protects completely against the development of hepatic necrosis a daily intake of 1 gramme of yeast protein allows 56% of the animals to develop the lesion Again on the diets with a carbohydrate basis 500 mg of casein daily are sufficient to prevent hepatic necrosis in all save 6% of the animals , if 500 mg of yeast protein are supplied instead the lesion develops in 88% of the animals

At first sight these results might be explained by postulating either that yeast contained a toxic substance or, alternatively, that it is deficient in some essential nutriment Our results show that the latter explanation is correct First, if yeast contained a toxic agent then one would expect that the greater the amount of yeast in the diet the greater the incidence, and the swifter the development, of the hepatic lesions In our experiments the reverse is true When the intake of yeast protein is about 500 mg per rat per diem 70% — 90% of the animals show necrosis between, on the average, the 45th and 60th days , when the intake is about 1 gramme daily only 56% of animals develop necrosis and then not until they have been taking the diet for an average of 100 days (Table II) Second, if yeast contains a toxic agent, then if we compare the incidence of necrotic lesions in a group of animals on a low casein diet (*e.g* 4% casein, carbohydrate diet, Table I), with the incidence in a group taking a diet containing the same amount of casein plus yeast protein (*e g* 4% casein, 4% yeast protein, carbohydrate diet, Table 2) we should expect the incidence to be higher in the latter group Actually we find that the incidence is lower on the diet containing yeast Thirdly, hepatic necrosis can be produced on diets which contain no yeast provided the casein content is sufficiently low

The conclusion from these experiments is, therefore, that dietary hepatic necrosis results from the deficiency of a substance which is either a component of, or associated with protein, and that casein is relatively rich whilst yeast protein is relatively poor in this substance

Our results with diets containing yeast are in conflict with the views of Gyorgy and Goldblatt (15, 16, 17) They believe that yeast protects against the development of all types of hepatic injury due to diet On examination of their data, however, it will be found that they base their opinion on experiments in which the yeast was *added* to the diet Thus addition clearly introduces not one, but two variables into the experiment, first an increase in total dietary protein, and second a possible specific protective action of yeast Our experiments show clearly that, when the total dietary protein is kept constant by *substituting* yeast protein for casein, the incidence of necrosis increases and they thus disprove the suggestion that yeast has a protective action against the development of hepatic necrosis This leaves, however, a further question to be considered if yeast protein is, in itself, relatively ineffective in protecting against necrosis of the liver, why does its addition to, in contrast to its substitution in, the diet decrease the incidence of this lesion ? Some light on this question is thrown by our experiments

On a 4% casein, carbohydrate diet (Tables 1 and 3) the incidence of hepatic necrosis is 57% On a 4% casein, 4% yeast protein carbohydrate diet (Table 2) the incidence is 21% There are two explanations of these results The first is that owing to the effect of yeast in stimulating the appetite the amount of casein eaten by the animals on the 4% casein, 4% yeast protein, carbohydrate diet was greater than that eaten by animals on the 4% casein, carbohydrate diet and thus these latter animals took in more of the protective protein (*cf* Table 1 and Table 2) The second explanation is that whilst yeast protein is inefficient as a protection against the development of necrosis of the liver it may be perfectly efficient for other biological purposes and thus its incorporation in a diet containing small amounts of casein would allow the body to make a more discriminative use of the casein available to the benefit of the liver The results on the fat diet raise similar problems On a 4% casein, fat diet (Tables 1 and 3) none of the animals developed necrosis, on a 4% casein, 4% yeast protein, fat diet (Table 3) 54% showed this lesion Again there are two possible explanations First that the inclusion of yeast, by increasing appetite, raised the intake of casein so that the amount consumed is lifted above the critical level of 200 mg per rat per diem into the range where necrosis develops Second, that the yeast, by being available for general purposes, removes the need for the breakdown of body protein and so prevents the liberation within the body of protecting substances which, on the low casein diet, had been supplementing the meagre supply of protective substance derived from the small amount of dietary casein The data at present available do not allow a decision to be made between these two explanations but the explanation postulating a " casein-sparing " action of yeast protein has the merit of

being compatible with the hypothesis required to explain the absence of necrosis during starvation and in animals receiving the 4% casein, fat diet

Influence of carbohydrate and fat on the protein requirements

It has long been known that susceptibility to certain liver poisons can be increased by an increase of dietary fat and decreased by an increase of dietary carbohydrate (26)   It might therefore have been expected that the amount of fat or the amount of carbohydrate in the diet would influence the liability of animals to develop dietary hepatic necrosis   At first glance our results might be interpreted as lending support to this expectation Thus on the 4% casein, 4% yeast protein fat diet 54% of the animals show necrosis , on the 4% casein, 4% yeast protein carbohydrate diet only 21% have the lesion   Inspection of the figures for food consumption on the two types of diet shows, however, that the appetites differed with the result that, although the proportion of protein might be the same, the amount consumed was different   Thus the daily amount of protein (i e casein plus yeast protein) consumed on the two diets mentioned was 532 mg and 608 mg respectively   Our results therefore provide no evidence that a high content of dietary fat facilitates or a high content of dietary carbohydrate protects against the development of hepatic necrosis   They indicate that the sole dietary factor determining the incidence of necrosis is the protein fraction of the diet

Influence of fatty infiltration of the liver

Many of the papers dealing with dietary hepatic injury attribute importance to fatty infiltration of the liver as a predisposing factor (5, 7, 30) Thus to quote from one paper "Experimentary dietary hepatic injury (diffuse or focal necrosis and cirrhosis in rats, with or without ascites and pleural and pericardial effusion) is determined by the dietary factors instrumental also in the production of fat infiltration of the liver   " (17) In this section our concern is to determine the relationship of fatty infiltration to massive hepatic necrosis, and to its sequel post-necrotic scarring, but, owing to the failure of previous workers to differentiate this latter condition from diffuse hepatic fibrosis, it appears that no evidence on this relationship has hitherto been produced

Table 3 shows the relationship between the fat content of the liver and the incidence of massive hepatic necrosis   It will be seen that the highest incidence of necrosis is in those groups of animals in which the amount of fat in the liver is either not, or only very slightly, raised   For example, after 66 days on a 50% fat diet those animals whose diet contains 16% casein had 27 18% of fat in the liver , those whose diet contained 7 2% yeast protein had only 6 01%   In the former group none of the animals showed hepatic necrosis , in the latter this lesion was present in 71%   Evidence to this same effect is provided by the other data, and

TABLE III

Showing the relationship between the fat content of the liver and the development of lesions in the liver

| Casein % | 10% | 8% | 8% | 8% | 0% | 0% | 0% | 4% | 4% | 2% | 2% | 4% | 4% | 4% | | | | |
Yeast protein %												3.6%	3.6%	3.6%	7.2%	7.2%	7.2%	14.4%
Choline mg/100 g of food				50			50		50		50			50			50	
Fat diet *Percentage of fat in liver (wet weight)*																		
34th day	24 25	25 12	5 10	—	—	—	—	—	—	—	—	9 10	—	—	6 52	—	—	4 83
60th day	27 18	25 74	15 35	—	—	—	—	—	—	—	—	8 70	—	—	6 01	—	—	6 38
Incidence of lesions in liver																		
Necrosis of liver (% of animals)	0%	9%	38%	80%	0%	0%	0%	0%				54%			71%	100%		56%
Diffuse fibrosis (portal cirrhosis) of liver	+	+	0	0	+	+						0			0	0		0
Early death No lesion of liver	0	0																
Carbohydrate diet *Percentage of fat in liver (wet weight)*																		
41st day	5 16	8 85	5 83	—	4 61	6 68	8 74	—	10 67	8 79	—	5 42	9 12	—	6 70	8 33		
76th day	7 05	4 47	5 81	—	6 08	8 37	—	14 96	10 72	—	4 32	4 50	—	—				
128th day	17 77			—	—	—	—	4 22	—	—								
Incidence of lesions in liver																		
Necrosis of liver (% of animals)	0%	6%	10%	43%	20%	57%	57%	25%	*0%	21%	10%		88%	89%				
Diffuse fibrosis (portal cirrhosis) of liver	0	0	0	0	0	0	0	0	0	0	0	0	0	0				
Early death No lesion of liver	0	0																

The spaces in the columns which are left blank opposite the heading "diffuse fibrosis of the liver" indicate that all animals died too soon or none have yet been observed for long enough, to allow an opinion to be expressed as to whether the particular diet causes the development of this hepatic lesion

0 in the same cross column indicates that no diffuse fibrosis has been observed in animals surviving for 250 days

— indicates that this particular experiment or estimation was not done, or that all the animals in this group died before the 250th day

* Only four animals in this group

further, there is even an indication that fatty infiltration actually protects against the development of hepatic necrosis   Thus on an 8% casein, fat diet, the percentage of fat in the liver was just over 25% and out of 33 animals on the diet, two showed partial and one extensive necrosis   When choline was added to the same diet to reduce the amount of liver fat, the percentage of fat in the liver rose only to 15%, and five out of the thirteen animals on the diet died with extensive hepatic necrosis

The beneficial effect of casein in protecting against dietary hepatic lesions (2) (8) has been attributed to its effect in reducing the amount of liver fat   It is evident from our figures that yeast is far more powerful in this respect than casein and yet necrosis occurs more readily on diets containing yeast than on diets containing casein   The protective action of casein against the development of dietary hepatic necrosis cannot, therefore, be explained by the lipotropic action of this substance

It thus appears that massive hepatic necrosis can, and usually does occur in the absence of fatty infiltration of the liver , that the protective action of casein in regard to this lesion is unconnected with its known lipotropic action , and that it is possible that a high content of liver fat may actually protect against the development of hepatic necrosis

The influence of choline

The concern which the majority of workers have shown in investigating the effect of choline upon dietetic injury of the liver in general is largely attributable to the importance attached to fatty infiltration of the liver as a factor predisposing to hepatic injury   If fatty infiltration is credited with this influence then obviously it becomes of importance to determine the effect of lipotropic agents, and of these choline has been established as one of the most powerful (1, 2, 3)   The concensus of published opinion appears to be that choline reduces the incidence of, or even prevents, the development of dietary " cirrhosis "   But again we are faced with the difficulty that the term " cirrhosis " has been used indiscriminately to include diffuse hepatic fibrosis and post-necrotic scarring, or even acute necrosis   It is, therefore, difficult to discover what was the actual effect of choline on any one of these conditions   The one lead is the statement of Earle and Victor (12) that the addition of choline to a diet which produced both " cirrhosis " and necrosis increased the incidence of necrotic lesions

In Table 4 are shown the effects of adding 4 mg of choline per rat per diem to the diets we have considered previously   On comparison with Tables 1 and 2 it is evident that choline does not reduce but rather increases the incidence of hepatic necrosis   Thus on the 8% casein, fat diet, one out of thirty-three animals died with extensive necrosis and two which were killed showed partial necrosis , on the same diet, when choline was added five out of thirteen animals died with extensive hepatic necrosis   Comparison of the severity of the lesions on diets with and without added choline gives the same impression

TABLE IV

Showing the effect of adding choline, 4 mg /rat/day, to the various diets on the development of massive hepatic necrosis

	FAT DIET			CARBOHYDRATE DIET				
Choline mg /100 g food	50	50	50	50	50	50	50	50
Casein %	8%	6%	4%	8%	6%	4%	—	4%
Yeast protein %	—	—	—	—	—	—	7·2%	3·6%
Food consumption per rat per diem (average over 11th to 40th day)								
(a) Food intake in grammes	5·91	4·52	4·23	7·78	7·69	7·01	7·6	7·77
(b) Energy value in calories	38·5	20·4	27·4	33·4	33·1	30·2	32·7	33·4
(c) Calories per 100 g body weight	32·4	20·8	26·2	24·6	28·9	20·0	30·8	26·5
(d) Protein intake in grammes	0·474	0·272	0·168	0·522	0·461	0·281	0·545	0·590
(e) Protein per 100 g body weight	0·398	0·276	0·176	0·458	0·405	0·241	0·511	0·469
Number of animals on diet	13	5*	5*	10	7*	7*	8	10
Necrosis of liver —								
(a) Total number and percentage (%)	5(38%)	4(80%)	0(0%)	1(10%)	2(29%)	4(57%)	7(88%)	1(10%)
i Extensive necrosis	5(38%)	3(60%)	0(0%)	0(0%)	0(0%)	3(43%)	5(63%)	0(0%)
ii Partial necrosis	0(0%)	1(20%)	0(0%)	1(10%)	2(29%)	1(14%)	2(15%)	1(10%)
(b) Time of death, in days, of animals dying with extensive necrosis								
i Average	95	48	—	—	—	50	43	—
ii Range	83–104	43–54	—	—	—	40–68	30–53	—

The explanatory footnote to Table I applies equally to this table Note that the addition of choline has little or no effect on the development of necrosis The exception is the fat diet containing 8% of casein The addition of choline to this increases the incidence and severity of necrosis amongst animals receiving the diet

* Data regarding further animals on these diets will be found in Table I

If, as we believe, choline tends to increase the incidence, and to aggravate the severity of dietary hepatic necrosis, the question arises as to how this effect is brought about   Our results on the relationship between the fat content of the liver and the development of necrosis suggest that a high content of fat in the liver may tend to protect the organ against necrosis   If this suggestion is correct then the administration of choline to animals whose livers contain much fat might, by reducing the amount of liver fat, allow necrosis to develop

Nutritional state of the animals in relation to the development of hepatic necrosis

It being well established that the requirements of animals for certain food stuffs varies with their nutritional state it is important to consider whether there exists any relationship between the incidence of hepatic necrosis in our animals and their state of nutrition   For this purpose attention may be directed to three factors   growth, food consumption, and the presence or absence of anæmia

There is no relationship between the growth of our animals and the development of hepatic necrosis   Thus, although the animals lost weight when taking many of the diets which produced necrosis (*e g* 7 2% yeast, protein fat diet), they gained weight steadily on others (*e g* 14 4% yeast protein fat diet)   Again our animals taking the diet which caused the greatest loss of weight , the 4% casein, fat diet, showed no necrotic lesions at all   Similarly there is no relationship between the calories consumed daily and the incidence of necrosis (Tables 1, 2 and 4)   Lastly none of our animals with necrotic lesions showed any significant degree of anæmia

Relationship of the composition of the diet to the severity of the hepatic necrosis and to the length of the latent period before its development

In the preceding sections we have considered only the incidence of necrotic lesions in the livers of animals on the different diets   Consideration of the severity or the speed of development of hepatic necrosis on the different diets adduces further evidence in support of the conclusions we have already drawn

Severity has been assessed by the extent of the lesion in the liver   When the necrosis extended over all lobes of the liver it was said to be severe , when it involved only certain parts of the liver, it was said to be mild   This classification accords with the clinical state of the animals for rats with extensive necrotic processes died, or appeared to be dying when killed, whilst rats with partial necrosis had only a moderate degree of illness and usually survived   Reference to Tables 1, 2 and 4 will show that the diets which produced the greatest incidence of necrosis in general also produced the greater proportion of severe lesions

In assessing the length of the latent period before development of the necrotic lesions we have had to confine our attentions to rats who died or

were obviously dying of hepatic necrosis   In effect this means that we have considered the latent period of development only in animals with severe lesions, for animals with partial necrosis had usually such a mild illness that we were not always certain from the clinical state whether necrosis had or had not developed   Again, however, the data in Tables 1, 2 and 4 show that the time required for necrosis to appear was less when the animals were taking a diet causing a high incidence of hepatic necrosis and greater when they were taking a diet causing a low incidence of the lesion

The question now arises why in a group of animals on a particular diet some had no lesions, some had only partial lesions and some had extensive lesions   The absence of lesions in some of the animals can be explained on the grounds that they were killed before the lesions had time to develop But the freedom from necrosis of other animals cannot be so explained In many groups there were animals who survived unscathed after several of their fellows had died of necrosis   The existence of such animals, and of those with partial necrosis, in a group in which the other members all showed extensive necrosis, can be accounted for, at least in part, by the method of group feeding which we used   All the animals in one group received their food out of one dish   As a result the more adroit animals got more food, perhaps sufficient to prevent their developing any necrosis at all , the less adroit got sufficient to prevent extensive necrosis but not sufficient to prevent partial necrosis, and the timid the least amount of food so that they developed extensive necrosis   The proportions of the different animals affected to these different degrees depends on the composition of the diet   Using the diet we have found most effective in producing hepatic necrosis, the 7 2% yeast protein, carbohydrate diet (Tables 1 and 4), we found that after the first death from hepatic necrosis every subsequent death revealed extensive lesions in the liver   With diets somewhat nearer normal composition the proportion of animals with extensive necrosis falls and the proportion with partial necrosis increases   And with diets still nearer normal a proportion of the animals survive without developing necrosis   It is thus possible by prescribing appropriate diets to produce at will either extensive or partial hepatic necrosis , or to give complete protection against the development of any necrosis

It may, therefore, be concluded that, not only the incidence, but the severity and the speed of appearance of massive hepatic necrosis is determined by the composition of the diet

Discussion on massive hepatic necrosis

The preceding results show that the incidence, the severity and the speed of appearance of massive hepatic necrosis are all determined by the quantity and quality of the protein in the diet   They further show that fatty infiltration of the liver is not a necessary antecedent of massive hepatic necrosis but may, on the contrary, serve as a protection against its development , that choline does not in any way prevent, but may even

facilitate development of the necrosis, and that massive necrosis of the liver can occur without the intervention of any exogenous toxic agent, be it chemical or infective, and solely as the result of a deficient diet   These findings throw light on several puzzling clinical and pathological features of the condition

The existence, before the appearance of massive hepatic necrosis, of a latent period during which the animal is continuously exposed to the effective dietetic conditions is explained as follows   At the beginning of the experiment the animal possesses certain stores of labile body proteins (36) Massive hepatic necrosis is due to a deficiency of certain constituents of protein   The latent period is the period of time required to deplete the body's stores of protein of the essential protein constituents   It will readily be seen that the more deficient the diet in these constituents the sooner the stores of protein will be depleted and the shorter will be the latent period

Our results also offer an explanation of the particular distribution of the necrotic lesions in partial massive necrosis of the liver   Protein is digested in the small intestine and the products of its digestion are carried to the liver in the blood of the superior mesenteric vein   This goes predominantly to the right lobes of the liver   As a result constituents of the superior mesenteric vein blood reach the left hepatic lobes only through the general circulation and after they have run the gauntlet of the liver cells of the right lobes Thus greater amounts of these constituents of protein required to prevent the development of massive hepatic necrosis reach the right lobes of the liver than reach the left   If therefore, the animals diet is grossly deficient in the necessary protein constituents the liver cells in all lobes will obtain too little and, when the requisite depletion of the stores of protein in the body has occurred, a generalised massive necrosis of the liver will result If, however, the diet supplies the protective factor in amounts larger, but still insufficient, for the needs of the whole liver, then the cells of the right lobes will obtain sufficient while the cells of the left lobes will get insufficient The result will then be that necrosis develops in the left lobe of the liver only

It remains to explain why animals who survive an attack of hepatic necrosis, may continue to take the offending diet and even regain a moderate degree of health   In many cases animals survive an attack of partial hepatic necrosis only to die later in a second attack of necrosis which involves the whole organ   In others a second attack of necrosis does not supervene and at autopsy, many weeks after the original illness, all that is found is scarring of the left lobes of the liver whilst the right lobes appear healthy   The course taken by the illness depends largely on the diet   If the diet is grossly deficient in the quantity and quality of protein the animal usually dies sooner or later of acute necrosis, if it is less deficient it may have one attack and then survive   Two explanations occur to us and each may apply under different circumstances   First when partial necrosis occurs it eliminates those cells which do not receive blood direct from the small intestine   The remaining cells now all receive directly blood which has the highest possible

concentration of the necessary protein constituent and none have to exist on blood which contains only a residual fraction   The continued healthy existence of the remaining liver cells may thus be assured   The second explanation applies particularly to animals on a grossly deficient diet who survive a first attack of partial necrosis   As a result of the failure of appetite in such animals the amount of protein consumed falls below the dietary level at which necrosis occurs, the animal mobilises sufficient first class protein from its tissues to prevent the further development of necrosis, and the illness is arrested   With returning health the appetite improves, sufficient dietary protein is consumed to eliminate the need for breaking down body protein, with the result that the liver cells are again exposed to a deficient supply of the necessary protein constituent and conditions are again ripe for a further attack of necrosis

III   THE PRODUCTION OF DIFFUSE HEPATIC FIBROSIS

The separate production of diffuse hepatic fibrosis

In the preceding sections several diets have been described which produce massive hepatic necrosis without producing diffuse hepatic fibrosis   In this section diets will be considered which produce diffuse hepatic fibrosis without producing massive hepatic necrosis   The two conditions have thus each been produced independently of the other and the proof of their essential difference established

Composition of the effective diet

The majority of the workers who have produced diffuse hepatic fibrosis by dietary means have used diets rich in fat (4, 5, 7, 12, 17, 27, 29, 33) We can confirm this finding for using our 8% casein, fat diet, or our 16% casein, fat diet, we have seen unequivocal evidence of diffuse fibrosis develop by the 150th day to 250th day of the experiment   But, as can be seen from Tables 1, 2 and 4, not all our diets containing the same amount of fat produced diffuse hepatic fibrosis in this time   No evidence of diffuse fibrosis was found in animals taking diets in which the dietary protein was entirely supplied by yeast, or in which the diet was supplemented by 4 mg of choline per rat per diem   It is thus clear that, although typical diffuse hepatic fibrosis can be produced in animals receiving a high fat diet, the effect of the fat diet in producing this lesion can be modified by the influence of other dietary constituents

Influence of fatty infiltration of the liver

On reference to Table 3 it will be seen that those of our fat diets which produced typical diffuse hepatic fibrosis (8% casein, fat diet and 16% casein, fat diet) also rapidly produced a marked fatty infiltration of the liver, whilst

the fat diets which did not lead to diffuse hepatic fibrosis did not produce any fatty infiltration of the liver   It thus appears that there is a close association between fatty infiltration of the liver and diffuse hepatic fibrosis and that factors which prevent, or delay the development of fatty infiltration also prevent or delay the development of diffuse hepatic fibrosis

In considering the relationship of fatty infiltration to diffuse fibrosis of the liver, attention must be paid to the time factor   After an animal has been given the appropriate diet fatty infiltration of the liver develops in a matter of days , diffuse hepatic fibrosis does not appear until months later It is therefore clear that fatty infiltration does not immediately cause fibrotic changes in the liver but that it must be present for a considerable time before diffuse hepatic fibrosis develops   In this connection an observation of Chaikoff and his co-workers (7) must be cited   They found, and we can confirm, that the fatty infiltration of the liver tends to decrease as the diffuse fibrosis develops   Whilst therefore the state of fatty infiltration appears to be a necessary antecedent to diffuse hepatic fibrosis it does not seem to be a necessary concomitant

Turning now to the factors which prevent the deposition of fat in the liver, casein, choline, and yeast, it is found that two of these are already well known for their lipotropic action   The lipotropic action of casein (2, 8) is relatively weak and it is only demonstrable where considerable quantities of casein are added to the diet   Nevertheless its action is not negligible as can be seen from Table 3 which shows the effect of small increments of dietary casein on the fat content of the liver of animals taking a carbohydrate diet   It is thus of significance that whilst we were able to produce typical diffuse hepatic fibrosis with a 50% fat diet containing 8% casein in 150 days it took 250 to 300 days to produce similar lesions with a 50% fat diet containing 16% of casein   Choline has a much more powerful lipotropic action than casein (2, 3, 8) but its lipotropic effect does not appear to continue indefinitely   It has previously been pointed out by one of us (H P H (18)) that, whilst choline will retard the deposition of cholesterol in the liver, this effect is not sustained and that eventually the livers of rabbits receiving a high cholesterol diet contain the same amount of cholesterol whether they have been given choline or not   This same waning lipotropic influence of choline is seen in the case of rats taking an 8% casein, fat diet and receiving a supplement of 4 mg of choline per rat per diem (Table 3) It is therefore possible that the action of choline in relation to diffuse hepatic fibrosis may be to delay rather than prevent the lesion   The lipotropic action of yeast appears to be the most powerful of the three   For two reasons it does not seem to us that it is possible to refer its lipotropic action to the choline content of yeast   First, our yeast did not supply as much choline, on any diet, as was supplied by a supplement of 4 mg of choline per rat per diem , and yet its lipotropic action was greater   Second, the lipotropic action of yeast showed no signs of waning throughout the period of our experiments   The conclusion appears to be that yeast contains an unknown

and very powerful lipotropic agent and that it is perhaps to this that the complete freedom from any trace of diffuse hepatic fibrosis of our animals on diets containing yeast, can be attributed

Although we refer to the lesion under discussion as diffuse hepatic fibrosis it would be incorrect to understand from this that the lesion at its inception, appears simultaneously throughout the whole liver mass The earliest lesions appear in the territory supplied by the left branch of the portal vein and even when the condition involves the whole organ the morbid processes tend to be more advanced on the left lobes This observation, however, should not lead to any confusion with post-necrotic scarring from which diffuse fibrosis is easily differentiated by the absence of necrosis and the uniformity of the fibrosis The reason for the more advanced state of the lesions in the left lobes can be seen from examining the livers of animals which have been taking the diet for only a short time It is there seen that the left lobes of the liver are far more heavily infiltrated with fat than are the right lobes This we attribute to the right lobes receiving a relatively more liberal supply of lipotropic factors through the blood from the small intestine Later this partition of fat between the two sides of the liver disappears and the whole organ becomes uniformly infiltrated But it is clear that at any time in the course of the development of diffuse hepatic fibrosis the left lobes of the liver will have been subjected to the influence of heavy fatty infiltration for a longer time than the right It is therefore only to be expected that diffuse hepatic fibrosis should commence, and tend to be more developed, in the left vascular territory of the liver

It would thus appear that diffuse hepatic fibrosis produced by dietetic means results from long continued, intense, fatty infiltration of the liver and that factors which reduce this fatty infiltration also retard the development of diffuse hepatic fibrosis

Discussion on diffuse hepatic fibrosis

In previously published reports on the effect of diet in producing fibrotic lesions in the liver no distinction has been drawn between post-necrotic scarring and diffuse fibrosis of the liver, both processes being included together under the term " cirrhosis " For this reason when trying to discover the findings of other workers on the effect of different factors on the development of diffuse fibrosis it is only possible to take account of those papers in which descriptions or photographs of the lesions produced establish that the lesions under consideration are in fact those of diffuse hepatic fibrosis and not those of post-necrotic scarring Applying this criterion it appears that undoubted examples of diffuse hepatic fibrosis have been produced experimentally by four groups of workers , Chaikoff and his colleagues (7) who produced it in dogs Rich and Hamilton (27) who produced it in rabbits, and Blumberg and Grady (5) and Webster (34) who produced it in rats It is possible, although not certain, that the lesions produced in rabbits and guinea pigs by Spellberg, Keeton and Ginsberg (30) were also of this nature

All these groups of workers used high fat diets   Rich and Hamilton showed further that the lesion was prevented by supplements of yeast, and Blumberg and Grady that it was prevented by choline   All the diets used by these workers were such as would produce fatty infiltration of the liver

Fatty infiltration of the liver can, however, be produced in two ways, either by giving a diet rich in fat or by giving a diet poor in lipotropic factors Thus fatty infiltration of the liver can readily be produced by giving a pure carbohydrate diet which is at the same time poor in lipotropic factors (2) Our 8% casein carbohydrate diet is of this nature and produces, as can be seen from Table 3, a moderate degree of fatty infiltration   Theoretically such a diet might be expected to lead to the development of diffuse hepatic fibrosis, but we have not observed unequivocal evidence of the lesion in any of our animals during the observation period of 250 days   It seems probable however, that Lillie, Daft and Sebrell (22) produced diffuse fibrosis by means of their first carbohydrate diet   This contained 82% of starch, 4% of casein and 5% of brewer's yeast and resulted in the development of diffuse fibrosis after ten months on the diet, in both young and old rats In later papers this group of workers claimed that by altering this diet they had improved their method so that now they could produce the lesion in 7 to 12 weeks (10, 11, 23, 24)   The alteration in the diet consisted of a reduction in the dietary protein by removal of the brewer's yeast and, from their description of the lesion resulting from this new diet, it appears very probable that the entirely different condition of post-necrotic scarring was now being produced   In our opinion it is highly improbable that diffuse hepatic fibrosis can be produced by dietetic means in rats in less than 150 days and we suspect that when claims are made to have produced the lesion more rapidly then the lesion in question was post-necrotic scarring

Most workers investigating dietetic injury of the liver have used diets, such as our 8% casein, fat diet, which produce both necrosis and diffuse fibrosis of the liver (4, 5, 12, 17, 24, 33, 34)   This is no accident   It having been suspected that the development of diffuse hepatic fibrosis was facilitated by a preceding fatty infiltration it was only natural to decrease the lipotropic factors in the diet devised   Of such factors protein was the most obvious The result was that diets were prescribed in which the content of casein was sufficient to prevent the animals dying and yet insufficient to retard the development of fatty infiltration of the liver   It is easy now to see why rats on such a diet would develop a variety of lesions   The animals least adroit in obtaining food would die of necrosis, those more adroit would survive to develop post-necrotic scarring, whilst the most adroit would live to develop diffuse hepatic fibrosis   Amongst animals on such a diet, who survived for any length of time, two kinds of fibrotic lesion in the liver would be found , simple diffuse hepatic fibrosis, and a mixed lesion in which the left lobes are the seat of post-necrotic scarring and the right lobes the seat of developing diffuse fibrosis   The natural conclusion from such experiments might well be that dietetic fibrosis of the liver was of one kind

only and that hepatic necrosis was but the first stage in the sequence to this lesion It is now apparent why such conflicting opinions have been expressed regarding the influence of substances like choline and yeast on dietetic " cirrhosis " of the liver for it is clear that the term cirrhosis of the liver has been used to include two distinct conditions and that choline, yeast and several other substances exert different effects on each of these

Thus it appears that diffuse hepatic fibrosis can be produced independently of massive hepatic necrosis Diets rich in fat, and diets devoid of fat but poor in lipotropic factors, can both lead to diffuse hepatic fibrosis These two types of diet possess the common property of producing fatty infiltration of the liver The development of fatty infiltration of the liver produced by either type of diet can be prevented, or its development retarded, by administration of lipotropic factors , and lipotropic factors have also been found to prevent or retard the development of diffuse hepatic fibrosis in animals taking these diets It, therefore, appears that fatty infiltration of the liver, of long duration, leads eventually to the development of diffuse hepatic fibrosis

SUMMARY

1 Two distinct lesions can be produced in the rat's liver by dietary means One, massive hepatic necrosis closely resembles the condition of " acute yellow atrophy " in man , the other, diffuse hepatic fibrosis, has an equally close resemblance to human portal cirrhosis

2 Massive hepatic necrosis is characterised clinically by an acute illness which develops suddenly, after a latent period of several weeks , pathologically by sudden necrosis of large areas of the liver Animals surviving the initial attack may, according to its severity, regain moderate health or die later with ascites, pleural effusions and oedema In either case scarring of the liver is found at the site of previous necroses and later nodular hyperplasia develops

3 Diffuse hepatic fibrosis is characterised clinically by a gradual deterioration of health extending over many months , pathologically by the slow development throughout the liver of a uniform fibrosis which first circumscribes and later invades the lobules

4 Massive hepatic necrosis is produced by diets low in protein Different proteins vary in their efficiency to prevent the lesion, indicating that the protective factor is a component of protein rather than an intact protein molecule

Our results provide no evidence that hepatic necrosis can occur from a vitamin or mineral deficiency, or that its development is influenced by the amount of fat or carbohydrate in the diet They suggest, however, that choline facilitates, while fatty infiltration of the liver protects against, its development

5 Diffuse hepatic fibrosis is produced by diets which, because they contain an excess of fat or are deficient in lipotropic factors, also cause fatty infiltration of the liver

6 It is concluded that massive hepatic necrosis, with its sequel nodular hyperplasia, is a deficiency disease due to lack of a component of protein whilst diffuse hepatic fibrosis results from long continued, heavy fatty infiltration of the liver

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Fig 1

Fig 2
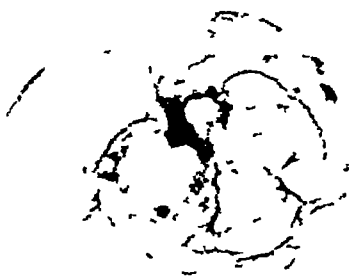
Fig 3

F g 4

Fig 1   Extensive massive hepatic necrosis   acute yellow atrophy stage   The necrotic processes are widespread throughout all the lobes of the liver   The dark spots on the surface represent petechial hæmorrhages   The other dark areas represent places where the necrotic process is a little more advanced than in the rest of the liver

Fig 2   Partial massive hepatic necrosis   subacute red atrophy stage   The dark portions of the photograph represent areas of necrosis in the stage of red atrophy   It will be seen that the process is confined to the left lobes of the liver   The rest of the organ is normal

Fig 3   Post necrotic scarring   commencing nodular hyperplasia in left lobes   The left lobes of the liver are cut up by deep and irregular scars   The right lobes are normal in every way (The appearance of flecking in the right lobes is due to hyperemia)

Fig 4   Diffuse hepatic fibrosis   portal cirrhosis   The whole surface of the organ is finely granular as a result of a diffuse fibrosis   There are no deep scars such as occur in post necrotic scarring

CARDIAC OUTPUT IN SEVERE ANÆMIA

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ACUTE reduction of blood volume is generally held to cause " shock," yet in post-hæmorrhagic and chronic anæmia levels as low as two litres may be found without decrease in systolic blood pressure (12)   These results called for further investigation of the factors maintaining the circulation   Using a direct Fick method in dogs, Blalock and Harrison (2) showed that cardiac output and the percentage utilisation of available oxygen in the blood were increased in anæmia following hæmorrhage   In man the main problem has been to find suitable methods for measuring cardiac output   Estimations by respiratory techniques (5, 10, 13, 14, 16) demonstrated increased cardiac output in anæmia, but the complexity of the methods makes accurate quantitative evaluation difficult and serial measurement impossible   Starr (17) obtained similar results with the ballisto-cardiogram, a method which may give valuable data on rapid changes but again may be criticised on a quantitative basis (4)   Cardiac catheterisation (3, 7, 11) affords a simple, safe and accurate means for determining cardiac output in man, and also allows direct measurement of mean right auricular pressure and percentage utilisation of available oxygen   This paper reports results obtained in post-hæmorrhagic and chronic anæmia, including some cases showing evidence of congestive heart failure, for which no cause other than anæmia was discovered

Methods

Observations were made about two hours after the midday meal   Cardiac output by a direct Fick method and mean right auricular pressure (R A P) were measured by cardiac catheterisation   The procedure and calculations are described elsewhere (11)   Average values for normal males in the supine position were —arteriovenous (A-V) oxygen difference 45 c c /litre and cardiac output 5 3 litres/min   Venous pressure (V P) was measured in an antecubital vein, and V P and R A P are expressed in cm of saline with the sternal angle as zero   While there is some individual variation when R A P is measured from a fixed point on the surface, we have usually found R A P to be about 4 cm below the sternal angle in normal supine subjects   Blood volume was measured by a concentrated

Case No	Sex Age	Weight kg	Position	Blood pressure mm Hg	Heart rate/min	Right auricular pressure cm saline above sternal angle	Hæmoglobin %	Blood volume litres	O₂ consumption cc/min	A V O₂ difference cc/litre	Cardiac output Litres/min	REMARKS
Post hæmorrhagic anæmia												
1	F 55	50	S	142/65	77	N	42 5	2 4	297	30	9 9	Hæmatemesis
2	M 23	66	S	112/50	104	—4	40 5	2 3	315	22	14 3	Hæmatemesis
3	M 43	65	S	130/40	96	—4 5	25	3 5	314	33	9 5	Hæmatemesis
4A	M 46	63	S	130/55	90	—3	68		310	23	13 4	Hæmatemesis
4B			S	104/80	68		71		265	56	4 7	10 days after 4A
5	M 51	61	S	104/50	110	N	24		275	37	7 4	Melæna
6	F 84	50	S	140/50	100	N	30		310	41	7 5	Melæna
Chronic anæmia												
7	M 70	42	T 45	110/50	88	O	21 2	2 3	245	31	7 9	Hypochromic anæmia Gastric carcinoma
8	F 67	56	S	140/55	100	N	30		279	32	8 7	Addison's anæmia
9	M 37	37	S	125/55	110	—3	32 5		231	30 5	7 6	Chronic hæmorrhage
10	M 50	56	S	120/58	106	—1	38 3	3 2	282	41	8 0	Addison's anæmia
11	M 67	52	T 45	114/50	84	—4	46 0	2 3	260	37	7 0	Addison's anæmia
Chronic anæmia with congestive heart failure												
12A	F 63	39	T 45	108/56	100	+20	12	4 0	250	19	13 1	Addison's anæmia and epistaxis
12B			T 45			+3*	35 5	2 6				4 days after 12A
13	F 62	63	T 45	116/50	112	+17	24	2 7	348	35	9 9	Hypochromic anæmia
14	F 77	44	T 45	135/70	72	+12	22 5	2 1	290	38	7 6	Carcinoma of bladder
15	F 55	49	T 45	106/40	96	+8	32	3 0	244	28	8 7	Acute hæmolytic anæmia
16	F 54	55	T 45	185/65	124	+4	24		330	31	10 0	Addison's anæmia
17	F 76	56	T 45	138/40	92	+4*	27 2	2 4	243	34	7 2	Addison's anæmia
18	M 77	60	T 45	120/50	70	+10*	14 7	2 2				Addison's anæmia
19	F 35	51	T 45	132/58	92	+8*	22 4	4 2				Addison's anæmia
20	F 45	54	T 45	120/60	100	+11*	29 4	1 8				Reticulosis
21	F 54	50	T 30	100/45	92	+1*	25	1 9	279	32 3	8 7	Lymphatic leukæmia

S = supine   T 45 = trunk elevated 45°   N = venous pressure within normal limits

* = venous pressure

corpuscle-hæmoglobin method (12) and hæmoglobin (100% normal = 15 5 g per 100 c c of blood) by a photoelectric method (9) Diastolic blood pressure was recorded at the first change in the sounds, in many cases sounds could be heard down to zero No treatment was given before the observations were made, other than rest and sedatives

Results

Results are shown in the table and some additional clinical data in the appendix All cases showed increased arterial pulsation, increased pulse pressure, and capillary pulsation in the finger tips The skin was pale but warm, many had " pistol-shot " sounds over the larger arteries, a positive Duroziez's sign and systolic murmurs on auscultation of the eyeball * Systolic and, less commonly, diastolic cardiac murmurs were heard Cases with congestive heart failure showed œdema, cardiac enlargement and electro-cardiographic changes These findings were occasionally present, to a less marked degree, in patients who showed no conspicuous rise of venous pressure

Post-hæmorrhagic anæmia Cardiac output was increased in all cases A second estimation in Case 4, ten days later, showed a normal output, though there was little difference in the hæmoglobin concentration on the two occasions Blood volume was reduced in the three cases in which it was measured The phase of increased pulse pressure appeared a day or more following the beginning of hæmorrhage Cases 2, 3 and 4 had an initial period of low blood pressure preceding the phase of increased pulse pressure

Chronic anæmia (with or without cardiac failure) Cardiac output was increased and blood volume reduced Patients with the highest venous pressure showed the lowest hæmoglobin concentration Case 12 had the lowest hæmoglobin and the highest venous pressure and cardiac output Blood volume, however, was not as low as in some cases Transfusion of concentrated corpuscles produced improvement as judged by the fall in venous pressure, noted 4 days later, when the blood volume was also found to be reduced Cases 13, 14, 15 and 20 ended fatally, the venous pressure remaining high until the end The table shows that resting minute oxygen consumption was not decreased in these cases of post-hæmorrhagic and chronic anæmia In some cases oxygen consumption was probably slightly increased

Discussion

In previous observations (11, 15) we have confirmed in man the work of Starling and Wiggers establishing that minute output is increased by raising the venous filling pressure on the right side of the heart, by accelerating the

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* These signs have been termed ' the hyperkinetic syndrome by Harrison (9) and ' hyperkinæmia by Starr (17)

heart, and by the action of substances, such as adrenaline, which affect the heart directly Since venous pressure and heart rate changes in man cannot be investigated separately, as in the heart lung preparation, they must be considered together Although our numbers are insufficient for statistical analysis, they suggest that R A P in post-hæmorrhagic and chronic anæmia is usually a high normal figure In normal subjects, increasing the heart rate to about 120 per min by atropine usually caused an increased cardiac output (11), though this was sometimes counteracted by a considerable fall in R A P (by 3 to 6 cm saline) The increased cardiac output in anæmia, which is of the same order as that produced by such doses of atropine, could result from a more moderate increase in heart rate combined with a high normal R A P This view is supported by the increased, often greatly increased, R A P in the most severe cases On any present clinical assessment a chronic increase of pressure in the right auricle at rest constitutes " congestive heart failure " But in these anæmic cases it is difficult to accept the suggestion that such increased R A P results fiom a passive damming back of blood behind a failing heart Although the heart may be failing to meet the demands made upon it on the venous side, it is still putting out twice oi thrice the normal minute volume In the presence of a reduced blood volume the rise of R A P must be the result of a physiological adjustment This adjustment is due to mechanisms as yet unstudied, but cleaily involving considerable reduction in the peripheral capacity of the vascular system Clinical observation of the pulse and skin circulation as well as calculation of total peripheral resistance (1) indicate peripheral arterial dilatation, so that reduction in vascular capacity must be at the expense of the capillary or venous systems It is possible, therefore, that greatly increased R A P represents the final stage of a continuous process of adjustment, which results in the maintenance of the minimum necessaiy minute output In the Fick method of estimating caidiac output, caidiac output in litres per min =

$$\frac{\text{Oxygen consumption in c c per min}}{\text{A-V oxygen difference in c c per litre}}$$

The figure for the denominator of this equation is limited by the oxygen cairying power of the arterial blood For example, if the hæmoglobin is 100%, arterial blood contains about 200 c c of oxygen per litre (allowing for temperature and pressure and assuming 95% satuiation) At a normal resting oxygen consumption of 250 c c per min , cardiac output cannot be less than $\frac{250}{200}$ = 1 25 litres per min But if the hæmoglobin is 10%, arterial blood contains only about 20 c c of oxygen per litre, and at the same oxygen consumption of 250 c c per min , cardiac output cannot be less than $\frac{250}{20}$ = 12 5 litres per min In Fig 1 the cardiac output data from the table and 9 normal subjects are plotted against hæmoglobin concentration

on a logarithmic scale   Two straight lines represent 100% and 25% utilisation of available oxygen, and it will be seen that, as hæmoglobin falls cardiac output tends to approach the theoretical minimum output Expressed in another way (Fig 2), the percentage of available oxygen removed in the periphery is increased   In the most severe cases of anæmia 80 to 90 per cent of available oxygen is removed and it is clear that, if this were not possible, cardiac output would have to reach even higher figures to maintain minute oxygen supply   Fig 1 shows that three of the post-
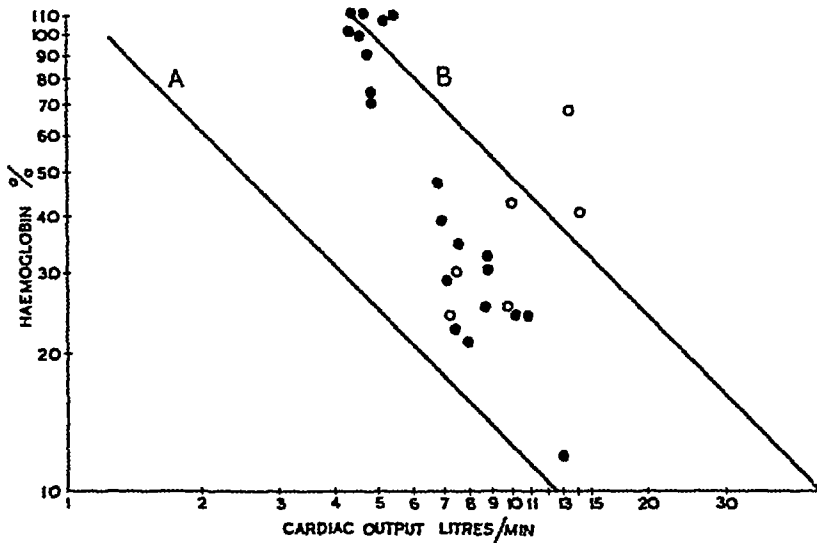
Fig 1   Cardiac output plotted against hæmoglobin concentration on a logarithmic scale   The nine points at the top of line B are data from normal subjects over the age of 40   the remaining points are obtained from the data in the table   Circles indicate post hæmorrhagic cases   Line A represents 100% utilisation of available oxygen at an oxygen consumption of 250 c c per min. (namely, the minimum possible cardiac output at different hæmoglobin levels)   Line B represents 25% utilisation of available oxygen.

hæmorrhagic cases had minute outputs conspicuously greater than the minimum necessary at the individual hæmoglobin level   This difference between acute hæmorrhagic anæmia and chronic anæmia requires further study   In view of the increased utilization of available oxygen in the resting state it is not surprising that ischæmic muscle pain is so easily induced in severe anæmia

Increased cardiac output and increased removal of available oxygen are two adjustments which serve to maintain minute oxygen supply   A third adjustment may be an active decrease in blood volume   At a given rate of flow, available oxygen depends on the concentration of hæmoglobin, so that when total circulating hæmoglobin is low, reduction in blood volume

increases available oxygen if it is attended by concentration of hæmoglobin The results in many cases suggest that reduction in blood volume is a more active process than a simple decrease of the number of red cells , thus in Case 12 concentrated corpuscle transfusion was followed in a day or two by a lower blood volume and clinical improvement
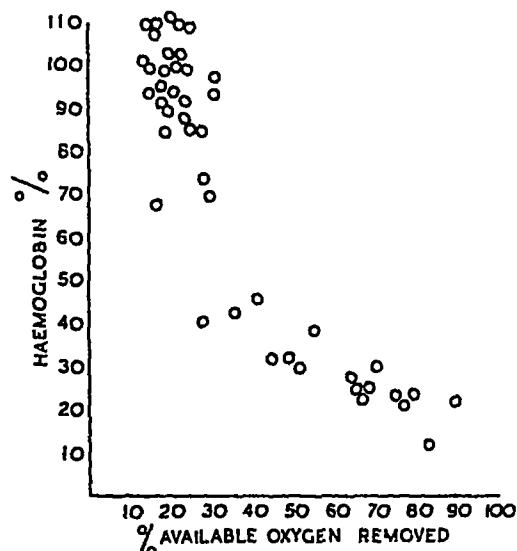
Fig 2   Shows increased peripheral utilisation of available oxygen at low hæmoglobin levels Resting normal and anæmic subjects

Dilution of hæmoglobin after bleeding is slow in man (6, 18) and changes after hæmorrhage give some indication of the time periods needed for these adjustments Rapid venesection of about 1 litre causes a fall in right auricular pressure and cardiac output (11), and we have never observed increased cardiac output *immediately* after controlled bleedings It appears therefore that an interval is required for the development of increased cardiac output and pulse pressure after hæmorrhage, and this phase may be preceded by a phase of low blood pressure and low cardiac output Yet blood volume levels in the phase of increased cardiac output are such that, if reached by rapid bleeding, they would result in a profound fall in blood pressure and cardiac output and might even endanger life As already indicated, adjustments producing the phase of increased cardiac output may include increased heart rate and the raising of R A P to normal Cases 1, 2 and 4 suggest that reduction of hæmoglobin concentration may not be the only stimulus, and the interval required for the development of increased cardiac output does not favour nervous mechanisms as the sole method of adjustment

SUMMARY

1 Resting minute oxygen consumption was not reduced in chronic and post-hæmorrhagic anæmia Oxygen supply was maintained by (a)

increased cardiac output which at the lowest hæmoglobin levels approximated the minimum necessary output, (*b*) increased removal of oxygen in the periphery (up to 90% of available oxygen), and (*c*) reduced blood volume, resulting in greater concentration of total hæmoglobin

2 Venous pressure is increased in the most severe cases Thus congestive heart failure in anæmia was associated with increased cardiac output and decreased blood volume Passive venous congestion will not explain these findings and conspicuous increase of venous pressure may represent the last stage of a process of adjustment, which maintains the necessary minute output

3 The phase of increased cardiac output and pulse pressure after hæmorrhage did not develop immediately and was preceded in three cases by a phase of low blood pressure and probably reduced cardiac output

APPENDIX.

Abbreviations C O = cardiac output, B V = blood volume, P = pulse rate, V.P = venous pressure sternal angle as zero, C T = arm to-tongue circulation time (sodium dehydro cholate) Electrocardiogram (E C G) T wave voltage in millivolts. Heart size T C.D = transverse chest diameter C T I = cardio thoracic index, C.A. = cardiac area.

Case 1 Hæmatemesis 3 days before admission. No faint 1st day B.P 135/85, blood urea 30 mg /100 c c 3rd day C O and B V measured, C T 9 secs.

Case 2 Hæmatemesis 10 hours before admission. Fainted. 1st day , B.P 105/85, P 100, Hb 76%, blood urea 87 mg /100 c.c 2nd day B P 120/80 3rd day further hæmatemesis B P 100/60 P 102 5th day B P 110/70 6th day B.P 120/30 8th day C O and B V measured

Case 3 Hæmatemesis 12 hours before admission Fainted 1st day B.P 90/75, P 116, Hb 58%, blood urea 98 mg /100 c.c., plasma protein 6 2 g /100 c.c 2nd day B.P 110/55 4th day B P 130/40 Hb 28%, plasma proteins 5 2 g /100 c.c.. 5th day C O and B V measured blood urea 33 mg /100 c.c., plasma protein 5 2 g /100 c c.. Treated B.P 125/75

Case 4 Hæmatemesis 6 hours before admission Fainted. 1st day B.P 60/30, P 80, 5 hours later B.P 85/60 P 80 blood urea 113 mg /100 c.c., plasma chlorides (as sodium chloride) 429 mg /100 c c., plasma proteins 4 6 g /100 c.c 2nd day B.P 100/60 to 110/70, P 84 to 96 3rd day C O measured, blood urea 84 mg 100 c c., plasma chlorides 435 mg 100 c c , plasma protein 5 1 g /100 c c 6th day B.P 115/60 blood urea 87 mg /100 c.c , Hb 50% plasma chlorides 625 mg /100 c.c., 9th day blood urea 45 mg /100 c.c.. 13th day C O measured Saline solution was given by mouth. No transfusion was given.

Case 5 Onset of hæmorrhage uncertain. Blood urea 216 mg /100 c c., plasma chlorides 551 mg 100 c.c Died 3 days later in status epilepticus (known epileptic) No post mortem

Case 6 Malæna 1 week

Case 7 R B C 2,100,000 Hb 21 5% Post mortem —Heart weight 240 g.. Slight athoroma of coronary arteries Careful inspection of the great veins in which the catheter had lain showed no antemortem thrombus in this and other cases which came to post mortem.

Case 8 B P 210/110. 5 years before Left ventricle slightly enlarged.

Case 9 History of progressive weakness over 4 months. Slow bleeding from duodenal ulcer

Case 10 C T 10 sec., E C G Q.R.S low voltage, T¹ +1, T¹¹ +1 T¹¹¹ 0 Heart size T C D 24 2 cm., C T.I 59, C.A 125 cm° Treated B.P 146/90, P 70 80, E C G T¹ 4 T¹¹ 6, T¹¹¹ 2 Heart size C T I 47, C.A. 93 cm²

Case 11 C T 11 sec., E C G normal

Case 12 6 months progressive weakness. 6 weeks recurrent severe epistaxis. Gross generalised œdema. Heart size T C.D 22 1 cm., C T.I 79, C.A. 164 cm² Hb rose from 12% to 20% immediately after first concentrated corpuscle transfusion. 4 days later Hb

was 35 5%, V P +3 cm and B V lower   13 days later Hb 40%, V P normal   B P 130/80

Case 13   Generalised œdema   R B C 2,300,000 per c mm   Colour index 44   Blood urea 25 mg/100 c c   Urea clearance 59% of normal   Concentrated corpuscle transfusion resulted in clinical improvement, but sudden death occurred 10 hours later   Post mortem — Oedema of lungs   Heart weight 380 g   Coronary arteries, pericardium and heart valves normal   Other macroscopic and microscopic findings were compatible with severe iron deficiency anæmia

Case 14   Hæmaturia   R B C 1,800,000   Hb 28%   Generalised œdema   E C G slight S T depression in lead II and III, otherwise normal   Post mortem   œdema of lungs   Heart weight 400 g   Slight atheroma of coronary arteries, pericardium and heart valves normal

Case 15   A case of acute hæmolytic anæmia of unknown ætiology   History of only 7 days illness   R B C 800,000, Hb 28%, reticulocytes 30%, W B C 11,000, plasma bilirubin 2 7 mg/100 c c, blood urea 83 mg/100 c c   Slight œdema of ankles   E C G S T II and III depressed, T waves absent   1 day after C O and B V measurement, Hb had risen to 48% and V P had fallen to +4 cm   2 days later E C G showed $T^1 + 1$, $T^{11} + 1$, T^{111} 0   The condition was unaffected by splenectomy and further transfusions failed to maintain Hb concentration   V P remained elevated   Post mortem —œdema of lungs   Heart weight 320 g   Coronary arteries, pericardium and heart valves normal

Case 16   History of angina pectoris and intermittent claudication   Oedema of back and ankles   C T 10 sec   E C G left bundle branch block   Treated   no angina or intermittent claudication

Case 17   Oedema of legs   C T 10 sec   E C G S T I and II depressed, T^1 1, T^{11} 1, T^{111} 0   Heart size   T C D 24 5 cm   C T I 68, C A 177 cm²   Treated   B P 150/75   C T 14 sec   Heart size C T I 55, C A 132 cm²

Case 18   Generalised œdema   E C G Q R S low voltage   T waves absent   Heart size T C D 29 0 cm, C T I 60, C A 164 cm²   Treated   E C G $T^1 + 3$, $T^{11} + 3$, T^{111} 0   Heart size   C T I 44, C A 114 cm²

Case 19   Generalised œdema   C T 7 sec   E C G slight S T II and III depression   T waves slight or absent   Heart size   T C D 26 1 cm, C T I 58, C A 122 cm²   18 hours after B V measurement   Hb 41%, V P +1 cm E C G   T waves slight or absent   Treated E C G $T^1 + 2$, $T^{11} + 1$, $T^{111} - 1$ Heart size   C T I 50, C A 101 cm²

Case 20   C T 9 sec   E C G S T depressed in all leads $T^1 - 1$, $T^{11} - 2$, $T^{111} - 1$ Heart size   T C D 24 cm   C T I 55, C A 112 cm²   Diagnosis established by biopsy   No post mortem

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THE PREVENTION OF EXPERIMENTAL MASSIVE HEPATIC NECROSIS BY METHIONINE

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IN a previous paper we have shown that massive hepatic necrosis produced by dietary means is a deficiency disease caused by the lack of a component of protein (5) In this paper it will be shown that a component of protein, methionine, prevents the development of the lesion

Our attention was directed to this amino-acid by previous findings First, we had found that in rats massive hepatic necrosis is readily produced by diets in which the protein is supplied entirely by yeast but only with more difficulty when it is supplied by casein (5) Casein is rich in methionine (1, 2, 6) , yeast protein is relatively poor in sulphur compounds (8) Second, Weichselbaum (10) had reported that rats given a diet poor in cystine developed ' hæmorrhages " in the liver and that these were prevented when either the cystine deficiency was remedied or methionine was added to the diet Third, it has been stated that methionine is one of the substances which will prevent " cirrhosis of the liver " developing in rats on a deficient diet (3, 4) Experiments were, therefore, planned first, to determine the amount of casein which, when included in the most deficient diet, would prevent massive necrosis, and second, to test if supplements of methionine in the quantities contained in that amount of casein would also protect It also seemed advisable to investigate whether substances like choline or cystine, which are said to influence the development of dietary hepatic " cirrhosis," also influence the development of dietary massive necrosis

Methods

The kind of rats used, the feeding and housing of the animals* and the histological and chemical methods employed have been detailed before All diets contained 3% of salt mixture and the same vitamin supplements as were given in previous experiments and, as before, 8 g of food were allowed daily to each animal (5)

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* We are indebted to Dr Charlotte Himsworth for the care of the animals

TABLE

Showing the incidence of massive hepatic necrosis on the different diets

Dietary supplements	CARBOHYDRATE DIET									FAT DIET				
Casein, 8% included in diet (Cas)	—	—	Cas	—	—	—	—	—	—	Cas	—	—	—	—
Methionine 20 5mg/rat/day (M)	—	—	—	M	M	—	—	—	—	—	M	M	—	—
Cystine 2 24 mg/rat/day (Cys)	—	—	—	—	Cys	Cys	Cys	—	—	—	—	Cys	Cys	Cys
Choline 4 mg/rat/day (Chol)	Chol	—	—	—	—	—	Chol	—	Chol	—	—	—	—	Chol
Number of animals in group	*4	*4	9	13	11	11	11	*3	*3	9	11	11	11	11
Necrosis of liver, total and per cent	4(100%)	3(75%)	0	0	0	11(100%)	11(100%)	3(100%)	3(100%)	0	†1(0%)	0	11(100%)	11(100%)
Extensive necrosis	4(100%)	3(75%)	0	0	0	9(82%)	8(73%)	0	0	0	1(9%)	0	1(9%)	5(46%)
Partial necrosis	0	0	0	0	0	2(18%)	3(27%)	3(100%)	3(100%)	0	0	0	10(91%)	6(54%)
Dying with extensive necrosis — Average survival in days	63	60	—	—	—	72	78	—	—	—	96	—	88	64
Range	46 71	47 60	—	—	—	64 78	62 97	—	—	—	—	—	—	54 70

Methionine, cystine and choline were added to the diet, casein was substituted for an equivalent weight of corn starch

* Further data concerning groups of animals on these control diets will be found in a previous paper (5)

† There are reasons for believing that the animal which died from necrosis in this group was inadvertently transferred from the next cage in which were animals receiving the fat diet plus cystine

Two basic diets were used   Each contained 8% of yeast protein , but one consisted largely of carbohydrate, the "carbohydrate diet," and the other contained a high proportion of fat, the "fat diet"   The composition of the carbohydrate diet was as follows —dried baker's yeast 18%, corn starch 73%, arachis oil 5%, cod liver oil 1%, salt mixture 3%   The composition of the fat diet was the same save that 50% of lard was substituted for 45% of corn starch and the 5% of arachis oil

The substances to be tested were either included in, or added to, these diets   Casein was included in the diet, in the proportion of 8%, by substitution for an equivalent weight of corn starch   The amino-acids or choline were added to the diet   *Dl* methionine and *l* cystine were used Casein can be regarded as containing, on the average, 3 2% of methionine and 0 35% of cystine (1, 2, 6)   Daily supplements to each rat of 20 5 mg of methionine or 2 24 mg of cystine are equivalent to the amounts of these substances taken daily by a rat consuming 8 g of a diet containing 8% casein   The supplement of choline was 4 mg per rat per diem

The following seven diets were constructed from each basic diet , basic diet alone, basic diet plus choline, basic diet including 8% casein, basic diet plus methionine, basic diet plus methionine plus cystine, basic diet plus cystine, and basic diet plus cystine plus choline

The experiment lasted for 100 days   At the end of that time all the surviving animals were killed

Results

These are shown in the table and can be summarised as follows — complete protection against the development of massive hepatic necrosis on either basic diet is given by including 8% of casein in that diet or by adding *dl* methionine in the amounts contained in that quantity of casein   Cystine, in the amounts contained in an 8% casein diet, has no influence on the development of the lesion, and methionine and cystine together protect as effectively as methionine alone   Choline, in supplements of 4 mg per rat per diem, gives no protection, and the effects of choline and cystine in combination do not differ from those of either alone   It thus appears that the protection afforded by casein can be adequately explained by the action of the methionine it contains

It has previously been shown that it is the amount, and not the proportion of protein eaten daily which determines whether massive necrosis will develop (5)   It is therefore necessary to know the amount of food eaten on the different diets   The food allowance was 8 g per rat per diem   All this allowance was eaten by all groups of animals on the carbohydrate diets Animals on the fat diets ate on the average 6 g per rat per diem and this did not vary significantly between the different groups of animals   The factor of differing food consumption does not, therefore, enter into our results

The only difference between the results on the two basic diets is that the lesions in animals taking the fat diets are on the whole less severe than those in animals taking the carbohydrate diets Thus out of 29 animals showing necrosis on the carbohydrate diet 24 had extensive lesions in the liver , of 29 animals with necrosis on the fat diet only 7 had extensive lesions, and of these 5 were receiving supplements which included choline These results are in accord with our previous experience, and estimations of the liver fat in these animals support our previous observations that the severity of massive hepatic necrosis is mitigated by heavy, fatty infiltration of the liver produced by dietary means The effect of choline in increasing the severity of the hepatic lesions on the fat diet, to a degree comparable with that seen in animals on the carbohydrate diet, may thus be associated with its effect in reducing the liver fat content in animals on the former diet to the levels in those on the latter diet

Discussion

If it were certain that the hepatic lesions noted by Weichselbaum (10) in rats on a cystine deficient diet were the same as those we have produced then our present results could be regarded as confirmatory of his The only mention he makes of this lesion, however, is the bald statement that hæmorrhages were found in the liver , and necrosis, not hæmorrhage, is the characteristic in our animals Nevertheless there are two points which make it possible that we were both dealing with the same lesion The diet he used was on the borderline of protein deficiency (9) and, if his animals' appetites were poor, massive necrosis might well result , there is a superficial resemblance between areas of massive necrosis, at one stage of their development, and subcapsular hæmorrhages Against this possibility is the fact that Weichselbaum found that cystine protected against his lesion whilst we found that it did not protect against ours , but in this connection it should be noted that he used much larger amounts of cystine than we did

Assessment of the results of later workers is rendered difficult by the fact that they have not distinguished massive hepatic necrosis and its sequel nodular hyperplasia from true portal cirrhosis, but have grouped both lesions together as cirrhosis of the liver Thus Gyorgi and Goldblatt (4), regard hepatic necrosis simply as a stage in the sequence to portal cirrhosis They find that cystine increases, whilst choline reduces, the severity of dietetic cirrhosis of the liver , that cystine and choline together, and also methionine in large doses, give considerable protection , but that methionine, in the doses we used, has no effect Daft and his colleagues (3), claim that choline retards the development of " cirrhosis," but not necrosis , that cystine in large amounts aggravates " cirrhosis " but prevents necrosis , and that large doses of methionine protect against both lesions They used a 4% casein diet and claim to have produced both necrosis and " cirrhosis " Their description of the latter lesion (7), and our experience with a similar

diet (5), however, suggest strongly that the fibrotic lesion they produced was post-necrotic scarring and not a true portal cirrhosis If this be so then the two conditions they distinguish are, in fact, simply different stages of the same process, a process, which our present results show, can be prevented by methionine

SUMMARY

1 Massive hepatic necrosis in rats due to a protein deficient diet is prevented by including 8% of casein in the diet or by adding *dl* methionine in the amounts contained in that quantity of casein, to the diet

2 The lesion is not prevented by *l* cystine, in amounts contained in an 8% casein diet, by a daily supplement to each animal of 4 mg of choline, nor by a combination of choline and cystine in these amounts The protective action of methionine is not antagonised by cystine when both are given in the quantities present in an 8% casein diet

3 The protection against the development of massive hepatic necrosis afforded by casein can be accounted for by the action of the methionine it contains

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OBSERVATIONS ON A CASE OF ORBITAL VARIX *

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THE clinical syndrome produced by orbital varix is characteristic and includes the dramatic phenomenon, transient severe proptosis Inflation of a pneumatic cuff placed round the neck causes the varix to fill and affords a unique opportunity of studying the mechanical effects of retro-bulbar swelling upon various ocular and palpebral physical signs The findings may be compared with those in orbital tumour, Graves' disease, arteriovenous fistula of the cavernous sinus and those produced experimentally by injecting wax into the retro-bulbar space post mortem (5)

The patient, a man aged 45, complains of protrusion of the right eye on stooping He first noticed this 3-4 years ago when bending down to read thermometers at his work There is occasional, slight aching pain in the eye

The position of the right eye varies with posture being slightly sunken (compared with the left) when standing or sitting upright, more prominent when lying flat and conspicuously proptosed when bending over to touch his toes The Hertel exophthalmometer readings in these positions are 12 mm , 16 mm , and approximately 25 mm respectively , the reading for the left eye (14 25 mm) does not vary significantly with posture Compression of the cervical veins with a pneumatic cuff causes proptosis to a maximum of about 18 mm (Fig 1) Concomitantly the eyeball moves about 2 mm laterally but not upwards or downwards With release of compression the eye rapidly recedes In 10 control subjects, constricting the cervical veins with a cuff at 40 mm of mercury caused a mean ocular protrusion of 1 3 mm (range 0 5 — 3 0 mm)

The acuity and field of vision, fundus, pupil and pupillary reactions of the right eye are normal, but maximal protrusion maintained for 5 to 8 seconds causes blindness , vision returns and improves as recession occurs and is normal some 10 seconds afterwards When fully protruded, the globe shows distinct pulsation, synchronous with the carotid pulse and of amplitude about 0 5 mm It is presumably transmitted from the ophthalmic or internal carotid artery

It is generally agreed that the presence of a varix in the orbit can be inferred from the foregoing signs, this has, in fact, been demonstrated at operation (1, 2) When, in the present case, the cuff is inflated to a pressure of 40 mm of mercury the volume of the venous swelling becomes maximal, the increase measuring some 17 5 c c It fills steadily at a rate of approximately 1 5 c c per second but the rate of emptying is less uniform
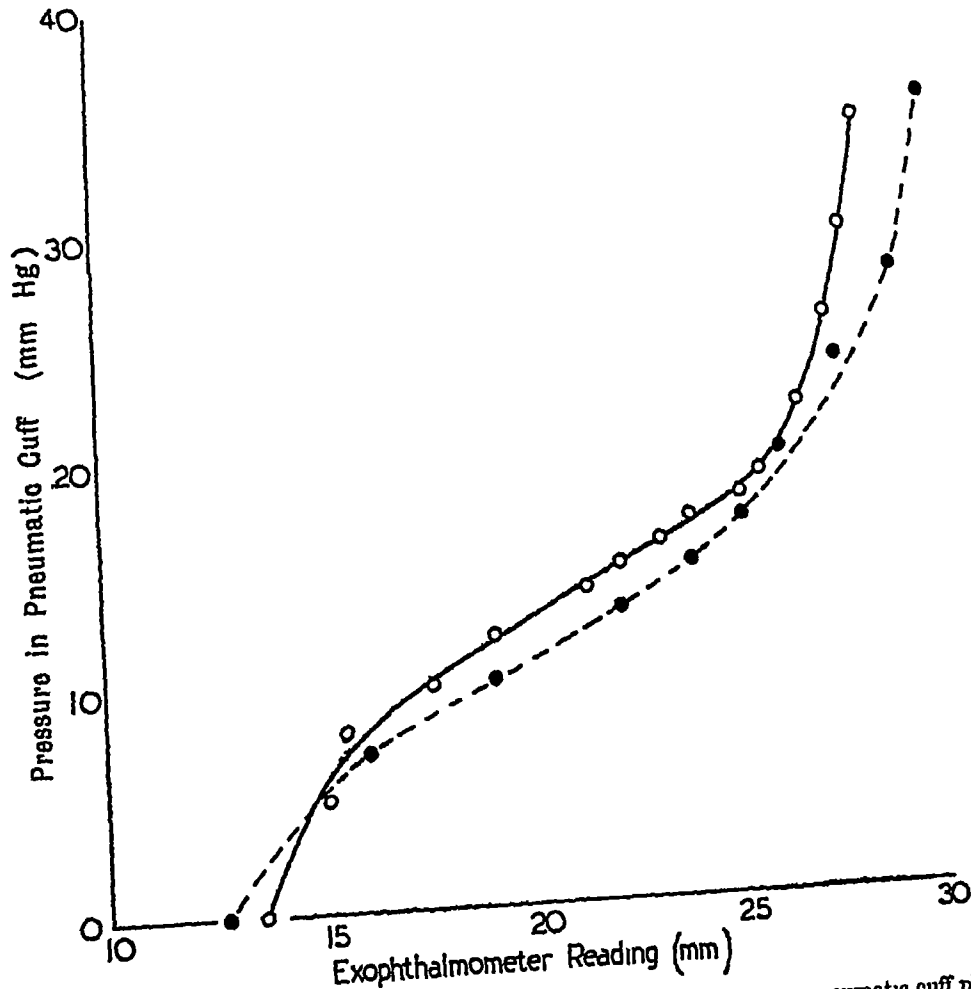
Fig 1   Protrusion of the eyeball as the varix is distended by inflating a pneumatic cuff placed round the neck   The cuff was removed and reapplied between the two sets of readings Under the conditions of the experiment the eye is not fully receded at the beginning, the true minimum reading being 12 0 mm

being at first faster, then much slower than this (Fig 2)   The mean orbital volume in male adults is 27 5 c c (4) so that, when fully distended, the varix occupies about two-thirds of the total capacity of the orbit   Recession of the eyeball and lids observed when the varix is collapsed indicates some atrophy of the tissues of the right orbit (5)

There is no evidence of varicosity of veins elsewhere
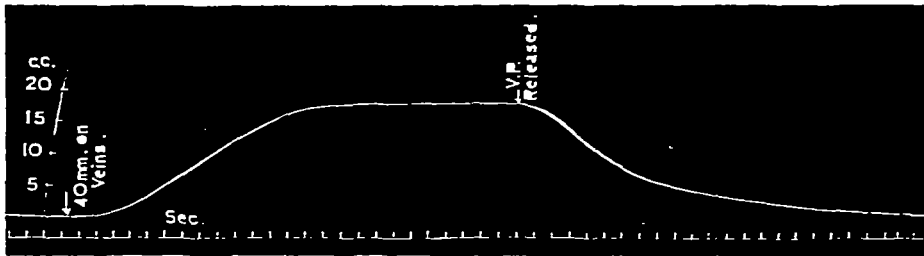
Fig 2   Measurement of volume of varix and rates of filling and emptying   To make the
record a special stent cup was built up and fitted over the eye, and connected to a float
recorder
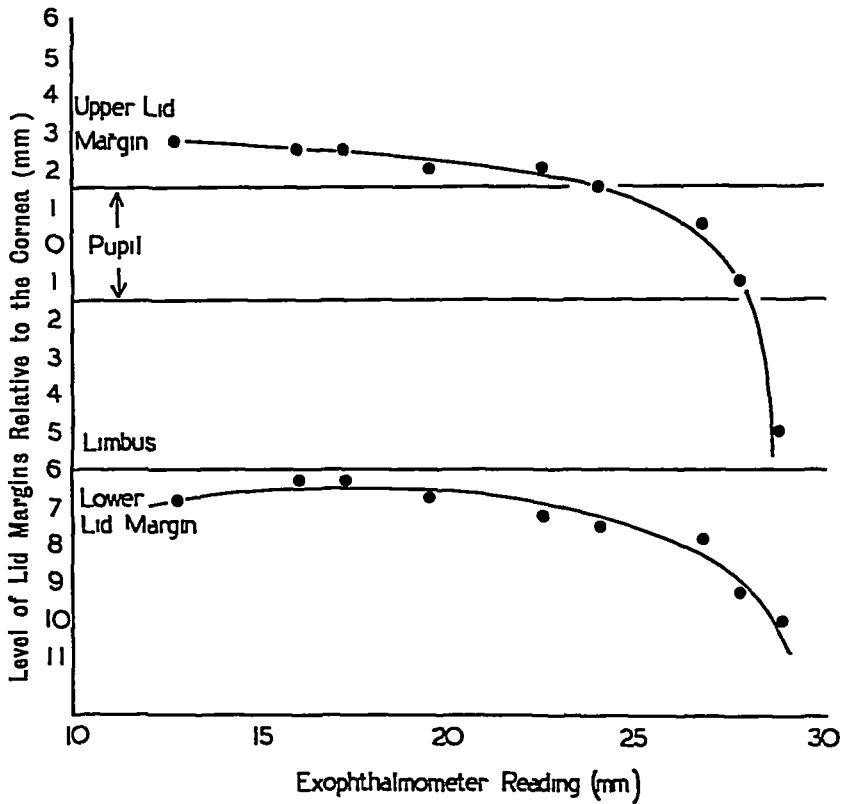
Fig 3   Changes in level of lid margins as the eye protrudes   Measurements are made in the
standard central position in which eye looks directly forwards   Reid s base line being
horizontal   Observed changes relative to the cornea represent true changes in level since
the horizontal plane of the globe remains constant throughout

Protrusion of the globe and lids as the tumour fills With the vault empty, the globe and lids, especially the upper, are sunken , a deep crevasse passes up, under the supra-orbital margin As it fills and exophthalmos develops, the lids first appear normal then progressively more bulged, until, when exophthalmos is maximal, both lids are conspicuously bulged This correlation between protrusion of the globe and lids is usual in conditions characterized by over-filling of the orbit The eyeball occupies only some four-tenths of the outlet , the orbital tissues round it protrude and so displace the lids forward (5) The lacrimal caruncle is also pushed forward , it broadens and in part protrudes through the palpebral fissure The conjunctiva becomes congested but does not prolapse

Changes in level of the lid margins as the eye protrudes are shown in Fig 3 Ptosis of the upper lid and retraction of the lower also occur when wax is injected into the retro-bulbar space post mortem Marked ptosis is usual in arterio-venous fistula Slight ptosis is common in orbital tumour but active contraction or spasm of the levator palpebræ may modify the changes dependent on purely mechanical factors in both tumour and Graves' disease

Exposure of a band of sclera between the margin of the lower lid and limbus occurs in exophthalmos and is often held to be a valuable diagnostic sign But up to 2 mm may be so exposed in normal subjects and in the present case this width is not exceeded until over 14 mm of exophthalmos develops Indeed at first the scleral band narrows Filling out of the rather sunken lower lid is associated with a distinct ascent of its free margin relative to the cornea , presumably this effect temporarily surpasses that of proptosis

With the eye sunken, apposition of the lids is imperfect on light closure, due to failure of the upper lid to descend fully Also, as the up-turned eye follows the observer's finger down, a band of sclera is exposed between the lid margin and limbus towards the end of depression These signs may result from the upper lid having to traverse the abnormally deep supra-bulbar crevasse At all events both phenomena disappear with the crevasse obliterated and the eye protruded , more of the lid is then available to cover the eyeball Such "mechanical" lid lag may be contrasted with that in Graves' disease produced by spasm of the levator palpebræ In the present case there is no crease in the skin of the upper lid as occurs when levator spasm is present (3)

Relationship between exophthalmos and eye movements (Fig 4) All movements of the eye remain full for the first 5 mm of the range and up to 10 mm , there is slight limitation of adduction only Thereafter generalized loss of movement occurs and in the end rapidly approaches completeness The expression "terminal mechanical ophthalmoplegia" may be used to denote this general restriction of movement with extreme orbital overfilling.

In Graves' disease, exophthalmos and ophthalmoplegia generally develop concomitantly and it has been claimed that the latter is a simple mechanical consequence of the former But the ophthalmoplegia shows important differences from that in the present case in respect of both its pattern and the degree of exophthalmos with which it is associated
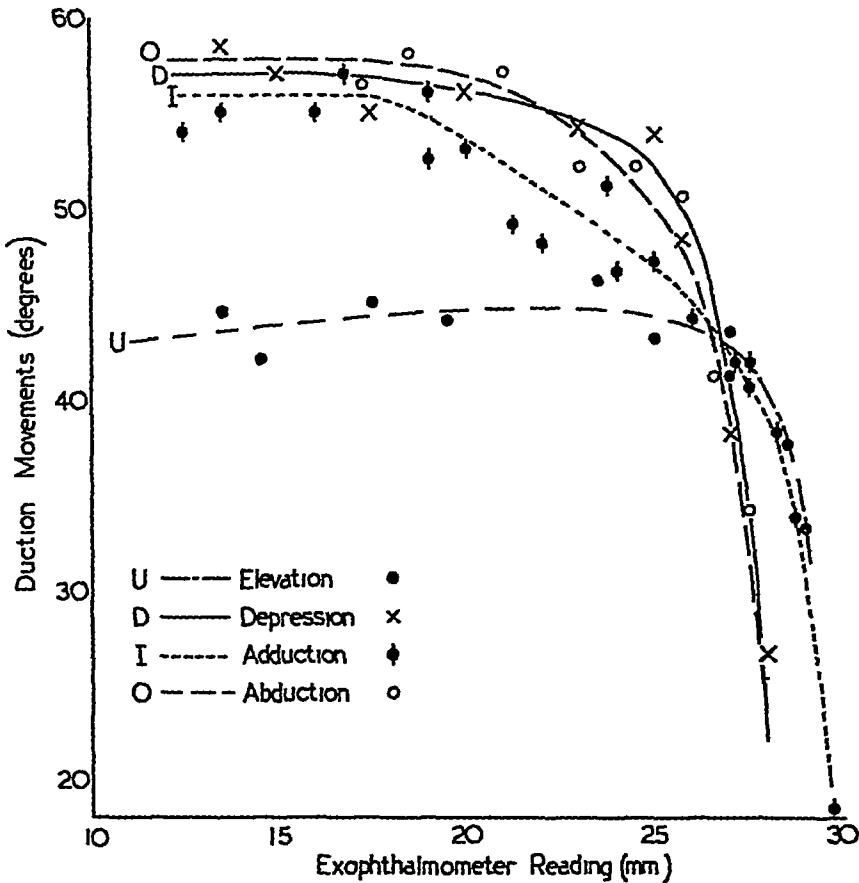
Fig 4   Range of duction movements from the standard central position as the eye protrudes

Thus in a group of patients with Graves' disease and well developed ophthalmoplegia, the average proptosis present was only 5 mm , in the present case no reduction in the range of eye movements was found until the eye had proptosed by 10 mm   Again in Graves' disease elevation is the duction most frequently and severely restricted , in the present case elevation was not reduced earlier or more severely than the other movements Orbital over-filling and the consequent proptosis may, when excessive,

contribute to the ophthalmoplegia of Graves' disease, but they are not the chief factors   It is more probable that the ophthalmoplegia depends on actual muscle damage, and a conspicuous increase in muscle fat has previously been reported (4)

The average proptosis in a group of 19 orbital new-growths was 9 mm with values up to 14 mm (6)   A state resembling terminal mechanical ophthalmoplegia occurs in large tumours   But in most tumours, there is no generalized loss of movement , paralysis appears to depend on (and so gives a clue to) the precise location of the tumour

SUMMARY

A case of orbital varix is described and used to examine the mechanical effect of retro-bulbar swelling upon various ocular and palpebral physical signs

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FAMILIAL IDIOPATHIC METHÆMOGLOBINÆMIA AND ITS TREATMENT WITH ASCORBIC ACID

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DEENY (5) recently reported on the treatment of two cases of polycythæmia vera with ascorbic acid   In a search for cyanosed subjects for further work, he came across two brothers, intensely cyanosed, who proved to be suffering from familial idiopathic methæmoglobinæmia   Ascorbic acid was given to one brother, and in a short time his colour became normal   We were then invited to investigate the second brother during treatment   Deeny, Murdock and Rogan (7) have described the cases and recorded our findings in brief   This further account is given because of the great rarity of the disease, these being the first cases of familial methæmoglobinæmia to be described in the British Isles , and because we wish to record work on the ætiology of the disease and on the mode of action of ascorbic acid

One brother, Case I (now aged 29 years), was blue from birth and the other, Case II (now aged 19 years), from an early age   Physical examination showed no cause for the cyanosis, and both enjoyed normal health apart from slight breathlessness, which did not prevent the younger from playing hockey for his local team

Methods

Skin colour was recorded at intervals during treatment by Mr Sidney Smith, who painted strips of canvas to match selected spots on the patient's skin   Comparison of these strips with Lewis's (19) standard colours was not satisfactory because the tint due to methæmoglobin is not the same as that produced by reduced hæmoglobin

Blood ascorbic acid was determined on whole blood by the method of Deeny, Murdock and Rogan (6)

Methæmoglobin was determined by three methods

    a   By difference between O_2 capacity and total pigments

    b   With a spectrocomparator

    c   By the method of Evelyn and Malloy (10)

Methods *b* and *c* were of service when the quantity of methæmoglobin in the blood became so small that determination by difference gave uncertain results

    a   Determination by difference O_2 capacity was found by duplicate analyses with the Van Slyke volumetric apparatus used according to the technique of Lundsgaard and Møller (23)   Total pigment was at first determined by the method of Rimington (26), in which all pigments are estimated as pyridine hæmochromogen   In our hands, the hæmin standards recommended by Rimington gave rise to some difficulty   The colour produced by a given amount of hæmin iron was less than that developed by an equivalent amount of hæmoglobin iron, while the tint of the final solution varied with the age of the hæmin solution   The intensity of colour of the pyridine hæmochromogen solutions was determined with a Hilger Spekker absorptiometer using a green filter transmitting maximally at about 550 mμ In our work 0 02 c c oxalated venous blood was measured into 8 c c $N/10$ NaOH, mixed and allowed to stand for a short time   (In a few cases parallel determinations using N/NaOH were made, but the stronger alkali did not affect the results)   2 c c colourless redistilled pyridine was then added and finally a few mg of a good specimen of sodium hydrosulphite dissolved in the mixture by gentle rotation   The strength of the hæmochromogen was measured in the absorptiometer and the blood pigment concentration read off from a calibration curve prepared by using normal blood of known oxygen capacity   This curve was checked at intervals During the course of this work, the alkaline hæmatin method of Clegg and King (3) for total pigments was published and was used for many of the later determinations   With both methods, all determinations were made in duplicate

    b   The spectrocomparator was independently devised by one of us, (D C H), and consisted in an arrangement which brought side-by-side the spectra produced by light passing through a known depth of a solution of the blood being examined (in which the normal pigment only had been previously converted into CO-Hb), and by light which had passed through the two cups of a two-stage colorimeter containing the same solution of blood in which all the pigments had been converted into met-Hb and CO-Hb respectively, (Clark and Gibson (2))   The cups of the colorimeter were then adjusted until the two spectra appeared identical, always keeping

the total depth of the two solutions equal to the depth of the unknown solution   The results by this method were in good agreement with the values given by the difference method

c   The method of Evelyn and Malloy (10) was modified to suit the Spekker absorptiometer by increasing the amount of blood used to 0 3 c c , laking by adding 10 c c water, then adding 2 c c M/5 phosphate buffer, (*p*H 6 9), and centrifuging to clear the solution

Methods used in investigating methæmoglobin reduction by enzymes of the erythrocytes   Venous blood from the patients and from normal subjects was defibrinated by stirring with Pt wire   The corpuscles were washed three times with a solution containing M/7 NaCl and M/55 phosphate buffer *p*H 7 4   About 50% of the total pigment was converted into methæmoglobin by treating the washed corpuscles for 10 min   at room temperature with 2½ times their volume of 0 05% amyl nitrite in phosphate saline   The corpuscles were washed thrice to remove excess nitrite and finally restored to blood volume with phosphate saline   2 c c portions of the suspension were transferred to the main chamber of Barcroft manometric flasks   The gas-space was filled with 20% CO, 80% N_2 mixture The course of the reduction of methæmoglobin to hæmoglobin could then be followed by the uptake of CO consequent on the combination of Hb with the gas   The manometers were shaken in a water-bath at 37°C Any traces of CO_2 were absorbed with KOH papers, and a correction applied to allow for the absorption of a small amount of CO by the KOH (Warburg *et al* (33))   Substrates were added from Keilin tubes

Results

Tests for methæmoglobin   Case II's oxalated blood had the chocolate tint of methæmoglobin   Samples diluted with water, and examined with the Hartridge reversion spectroscope, showed a strong absorption band in the red centred at 632-634 mμ This corresponds closely with the figure for methæmoglobin (630 mμ), and not with that for sulphæmoglobin (618 mμ) The pigment was entirely intracorpuscular, thus excluding methæmalbumin, which is always extracorpuscular and has a band at 623-624 mμ (Fairley (12))   On adding a trace of $Na_2S_2O_4$, or a few drops of $(NH_4)_2S$, NH_4OH or NaCN, the band in the red disappeared completely, confirming the presence of methæmoglobin and showing the absence of sulphæmoglobin

Treatment with ascorbic acid   The blood changes produced in Case II by treatment with 100 mg ascorbic acid twice daily for 16 days and subsequently with double the amount are summarised in Fig 1, the shaded area showing the amount of methæmoglobin   The skin colour recorded twice before and nine times during treatment showed a gradual disappearance of the cyanosis and its replacement by the normal reddish tint   The final

canvas however still differed slightly from a canvas painted from a normal person

The urine during the early stages of treatment showed only a faintly positive Schlesinger test for urobilin and urobilinogen and was free from bile pigment   No sugar, albumin or indican were observed   An unusual finding was the presence in every one of a large number of samples of urine tested of a chromogen which gave a deep rose-pink colour in Jaffe's indican test   The colour developed only on the addition of the oxidising agent,
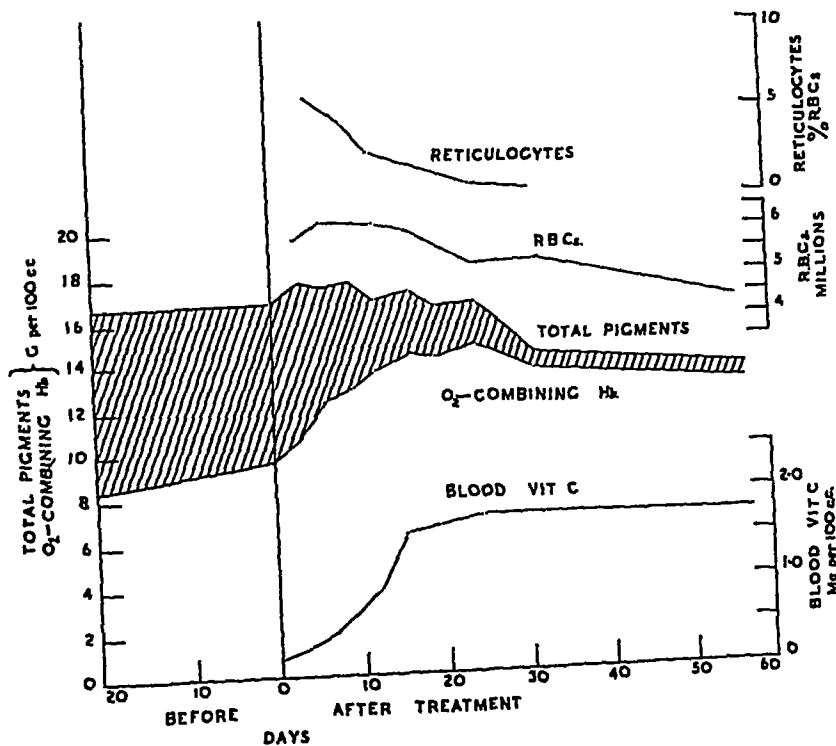
Fig 1   Blood changes in idiopathic methæmoglobinæmia during treatment with ascorbic acid

$Ca(OCl)_2$, $NaNO_2$, H_2O_2 or $FeCl_3$ The pigment, insoluble in $CHCl_3$, was extracted by amyl alcohol, then showing absorption bands at about 554 and 497 mμ The chromogen was not destroyed or removed by steam distillation or evaporation of the urine   (cf Fearon and Thompson (13)) It was probably not indole acetic acid, as a sample of this substance gave an absorption band further away from the D line, at 542 mμ The absorption spectrum and other properties make it probable that the chromogen was indole aceturic acid (Ewins and Laidlaw (11) , Homer (17)) This constant, strongly positive urorosein reaction appears to be rather uncommon, but it does not seem to be connected with the methæmoglobinæmia since it was not found in the urine of Case I

A curious feature of the urines of both Case I and Case II was the finding of streptococcus viridans (in large amounts) in every sample examined The streptococcus was still present a year after the beginning of treatment

Other findings in Case II during treatment were —Blood RBC 5,800,000 on 3rd day of treatment falling to 4 400 000 on 57th day Reticulocytes 5½% on 3rd day less than ½% on 31st day Mean corpuscular volume 78-97μ³ Price-Jones curve before treatment 5μ (5%), 6 (44%), 6½ (28%), 7 (15%) 7½ (3%) Fragility hæmolysis began in 0 4% NaCl W.B C 6 000 9 000 Differential count, normal Leishman film showed equal basophil staining Van den Bergh test normal direct and indirect (0 5 units) on 3rd and 9th days of treatment Blood culture sterile Alkali reserve, Van Slyke and Cullen (30) before treatment 61 c.c CO₂ at 41 mm. tension unchanged after 57 days' treatment Blood Met Hb, after 13 months treatment, 1 25g per 100 c c , (compare Case I, 1 3g after 10 months' treatment)

Action of ascorbic acid on methæmoglobinæmic blood in vitro It seemed desirable to see if ascorbic acid had any action in vitro on the blood of Case II to determine if reduction of methæmoglobin to hæmoglobin occurred, or if the methæmoglobin was removed by being destroyed, and if the intact red cell was necessary for the removal of methæmoglobin

Preliminary qualitative experiments with large amounts of ascorbic acid showed that while a decrease in methæmoglobin did occur, the reaction took place slowly and, in our experiments, was incomplete after several hours' aerobic incubation In one experiment, oxygen capacities were measured before and after incubation with ascorbic acid , the results, shown in Table I, indicate that methæmoglobin was being reduced to

TABLE I

Conversion of Met Hb to Hb by ascorbic acid in vitro

Addition	Hb (g /100 c c) by O₂ capacity		Increase in Hb (g /100 c c)
	Initial	Final	
NaCl	12 8	13 0	0 2
Ascorbic acid	12 8	15 7	2 9

Two 250 c.c conical flasks each contained 6 c c oxalated blood (total pigments 17 7 g /100 c c) taken from Case II shortly after the beginning of treatment 0 4 c c M/12 ascorbic acid, (brought to pH 7 2) was added to one flask and 0 4 c.c M/5 NaCl to the other, and the solutions incubated at 38° C for 2 hours with occasional shaking Initial and final O₂ capacities determined by the Van Slyke volumetric method

hæmoglobin rather than being destroyed The results of a second series of experiments, Table II, show that the reduction occurs after hæmolysis, and so does not depend on the presence of the intact red cell, and that, under our conditions, it is greater in the absence of oxygen, probably because autoxidation of ascorbic acid is prevented These results also demonstrate that ascorbic acid can penetrate the red cell, though the acceleration of reduction produced by hæmolysis suggests that the penetration is relatively slow

TABLE II

Conversion of Met Hb to Hb by ascorbic acid in vitro in presence of intact and hæmolysed red cells

Tube No	Additions	Final conc MHb (g /100 c c)	% reduction of MHb by ascorbic acid
1a	—	4 3	—
1b	1 mg ascorbic acid	2 9	33
2a	15 mg saponin	3 8	—
2b	15 mg saponin plus 1 mg ascorbic acid	2 0	47
3a	—	4 3	—
3b	1 mg ascorbic acid (anærobic)	1 8	58

Each tube contained 1 c c oxalated blood from Case II with 5 1 g /100 c c Met Hb in 17 7 g /100 c c total pigments Ascorbic acid, 1 mg in 0 07 c c (neutralised) Saponin, 15 mg in 0 1 c c The final volume was made up to 1 2 c c with 0 9% NaCl Tubes 3a and 3b were evacuated and all were then incubated at 37°C for 2 hours with frequent shaking The Met-Hb in aliquots of the final solutions was then determined by means of the spectrocomparator

TABLE III

Reduction of methæmoglobin by washed red cells from cases of idiopathic methæmoglobinæmia and from normal subjects

Subject	$\dfrac{\mu\text{mol MHb reduced} \times 100}{\mu\text{mol pigment iron} \times \text{hours}}$			Increase due to substrate	
	Cells	Cells +glucose M/100	Cells +lactate M/50	Cells +glucose M/100	Cells +lactate M/50
Case I	1 0	2 2	1 1	1 2	0 1
Case II	0 9	1 2	1 0	0 3	0 1
Normals					
H B	1 6	5 5	5 5	3 9	3 9
C L	1 1	4 9	5 6	3 8	4 5
Q H G	1 7	5 4	6 5	3 7	4 8
H A L	2 1	5 4	—	3 3	—
G D'R C	2 2	6 9	7 6	4 7	5 4
Av Cases I and II	1 0	1 7	1 1	0 7	0 1
Av Normals	1 7	5 6	6 3	3 9	4 6

Examination of the enzyme mechanism for the reduction of methæmoglobin within the corpuscles It has been shown by Warburg *et al* (32), that methæmoglobin is reduced in vitro by erythrocytes in the presence of glucose, while Cox and Wendel (4), have concluded that an enzyme process of this nature is probably responsible for the reduction in vivo of methæmoglobin formed by drugs We therefore tested the ability of the enzyme systems in the patients' RBC to reduce methæmoglobin formed by amyl nitrite

TABLE IV

Reduction of methæmoglobin in the presence of M/20,000 methylene blue by cells from cases of idiopathic methæmoglobinæmia and by cells from normal subjects

| Subject | $\dfrac{\mu mol\ MHb\ reduced \times 100}{\mu mol\ pigment\ iron \times hr}$ | | | Increase due to substrate | |
	Cells	Cells +glucose M/100	Cells +lactate M/50	Cells +glucose M/100	Cells +lactate M/50
Case I	1 3	68 0	1 6	66 7	0 3
Case II	2 3	62 5	2 3	60-2	0 0
Normals					
H.B	2 9	44 0	9 9	41 1	7 0
C.L	2 6	53 0	—	50 4	—
Q.H G	3 8	—	10 3	—	6 5
H.A L	2 2	—	9 6	—	7 4
G.D R C	4-6	43 0	15 4	38 4	10 8
Av Cases I and II	1 8	65 5	2 0	63 7	0 2
Av Normals	3 2	46 6	11 3	43 4	8 1

The results, given in Table III, along with figures obtained with cells from normal bloods under comparable conditions, show that while the normal cells respond to the addition of glucose or lactate with a considerable increase in the rate of reduction of the pigment, the rate of reduction by the pathological cells is almost unaltered The figures in the table correspond to the percentage of the total pigment which would be reduced in one hour For the patients, they are averages of closely agreeing duplicate determinations as are most of those for the normal controls Initially, about 50% of the total pigment was present as methæmoglobin In all cases the duration of the experiments was so arranged that considerable amounts of methæmoglobin remained unchanged at the end The results could be

explained by postulating either that the enzyme system in the pathological cells was deficient, or that the methæmoglobin *formed* in them was of an abnormal type which was not readily reducible   The finding that in the presence of glucose plus methylene blue, methæmoglobin is reduced in the pathological cells at least as fast as in normal cells is strong evidence against the second assumption, namely a difference in the type of pigment   It is probable that methylene blue acts as a carrier in this reaction by being reduced to leuco-methylene blue in the presence of glucose and an enzyme system, the leuco-methylene blue then reducing the methæmoglobin to hæmoglobin   (Table IV)

It has been found, further, that in normal cells, methylene blue catalyses reduction by lactate, while this catalysis does not occur with the pathological cells   As the results with glucose as substrate showed that leuco-methylene blue reduced the methæmoglobin present in the pathological cells, this result must be ascribed to a deficiency in the enzyme system normally responsible for reducing methylene blue in the presence of lactate   These findings are illustrated in Table IV   The precise interpretation of the results, however, must await more detailed information about the processes occurring in normal erythrocytes during the reduction of methæmoglobin

Discussion

Some points of interest concerning the present Cases I and II and other published cases are set out in Table V   The disease has now been established in three families, in two isolated cases whose histories strongly suggest that it was familial and in a further four isolated cases   Two of the isolated non-familial cases, namely Müller's (24) and Leiner and Minibeck's (18), have been included as idiopathic though they were described as enterogenous In both, the colour was congenital, and this is often a feature of the idiopathic form   It is noteworthy that the cases of Bensley et al (1) were brother and sister   Lian (20) used ascorbic acid in the treatment of one of their cases, and also were able to demonstrate reduction of the abnormal pigment in vitro   We were not aware of their results at the time our own work was carried out

The mode of inheritance of this disease is not yet clear, because the material so far available is limited to very few families   As regards the present family, in addition to the two brothers Case I and Case II, of 29 and 19 years respectively, the present generation consists of one sister, aged 22, and two brothers aged 24 and 26   There was another brother who died of whooping cough at the age of 9 years   The mother is living (aged 57) The father died (aged 61) from congestive heart failure and chronic bronchitis, his colour is stated by the family doctor to have been normal   Spectroscopic examination of blood samples from the mother and from all the surviving members of the present generation showed that with the exception of Cases I and II there was no trace of methæmoglobin   Thus two children

TABLE V

	Sex	Age	Age when cyanosis began	Total pigments (g per 100 c c)	O₂ combining Hb (g per 100 c c)	Met Hb (% total pigments)	R.B Cs (millions)	Reticulocytes (% R.B Cs)	Disability	Colour	Bowels
Hensley (1)	♂	77	11	19·0	11·6	0	5·3	—	Nil	Bluish	Constipated
	♀	43	11	11·4	13·7	5	4·5	—	Nil	Cyanosed	Constipated
Jann (20)	♂	26	0	16·7	9·2	15	5·0	1·5	Dyspnœa, headache	Dark violet or dirty blue	Normal
	♂	28	0	18·0	10·1	35	5·0	—	Dyspnœa in severe exercise	Ditto, but less so	Normal
Deeny (7)	♂	20	0	—	—	—	5·0	—	Dyspnœa in moderate exercise	Livid blue	Constipated
	♂	19	0·11	19·0	9·0	12	5·8	5·5	Nil	Face slate blue	Normal
Hitzenberger (10)	♂	23	0	18·7	11·8	10	5·8	0·0	Nil	Blue yellow undertone	Normal
van Thienen (31)	♀	13	0	—	—	—	5·3	—	Nil	Pale livid (bluish london)	Normal
Miller (21)	♀	9	0	—	—	—	4·0	—	Nil	Blue with lead or steel tinge	Constipated
Deeckmann (8)	♀	27	0	—	12·8	30	4·5	—	Nil	Blue	—
van Tjer (29)	—	19	0	—	—	—	—	—	Nil	—	—
Leitner and Mininski (19)	♂	08	0	15·7	13·8	17	5·8	—	Nil	Intense blue black	Loose

Familial cases · Familial cases · Isolated cases

out of six were affected, the parents both apparently being free from the disease Careful enquiry failed to disclose any consanguinity between the parents, nor was there any history of cyanosis in relatives Neither of our two cases nor their brothers or sister are married The following relevant facts are available from published records No other relatives of the two cases of Bensley (1) were cyanosed The female case had eight normal children It was said that the mother of the two cases of Lian (20) had had an abnormal complexion at the age of eleven, when examined, her blood did not contain methæmoglobin She had 10 children, 6 boys and 4 girls, 5 of them, 3 boys and 2 girls, were said to have born blue, but of these one boy had died at 18 months of diarrhœa, one girl at 5 years of whooping cough, and the other girl at 18 months of measles One of their cases had a single normal child Hitzenberger's (16) case apparently had had two brothers with similar abnormalities The condition may be associated with other hereditary defects thus Hitzenberger's case was dwarfed and mentally deficient, Lian stated that in their patients the mean diameter of the RBC and the mean corpuscular volume were significantly greater than normal, while a low blood fibrinogen afforded evidence of possible congenital liver defect Consideration of the evidence as a whole makes it seem probable that the disease is inherited as a recessive character, while the cases of Bensley (1) indicate that it may show itself in either sex

Aetiology of idiopathic methæmoglobinæmia There appear to be three kinds of methæmoglobinæmia (1) Methæmoglobinæmia due to drugs such as acetanilide, antipyrine, phenacetin This is the commonest type of methæmoglobinæmia The methæmoglobin generally disappears from the blood 24-72 hours after stopping the drug

(2) Enterogenous methæmoglobinæmia, which occasionally occurs in adults with marked diarrhœa, and is possibly caused by excessive absorption of nitrites from the gut The pigment is intracorpuscular van den Bergh and Grutterink (28), Lichtenbelt (21), and Dunævskiy and Kozlovskaya (9), describe a total of 8 cases In some of the cases of van den Bergh and Grutterink, the RBC contained an abnormally large amount of nitrite

(3) Idiopathic methæmoglobinæmia In this variety, there is constant cyanosis of a bluish tint, usually congenital, occasionally familial The general health is good The blood shows substantial amounts of intra-corpuscular methæmoglobin and a tendency to polycythæmia

Peters and Van Slyke (25), have suggested that methæmoglobin is constantly being formed in normal persons, and that it is reduced by enzyme systems within the corpuscles Accumulation of methæmoglobin might be due to

(a) An abnormally rapid rate of formation, exceeding the capacity of the normal mechanisms for removal

(b) A deficient mechanism of removal with a normal rate of formation

(c) The presence of an abnormal type of methæmoglobin not reducible by the normal mechanisms

Drug methæmoglobinæmias probably belong to type (a), as shown by the rapid disappearance of the pigment on withdrawal of the exciting cause, while van den Bergh and Grutterink's (28) observation that the pigment disappeared in 24-48 hours when their cases of enterogenous methæmoglobinæmia were given a milk diet, suggests that this condition has a similar origin   The observations which have been made on our cases indicate that there is a deficiency of the enzyme mechanism for the removal of methæmoglobin   It is not possible to say whether this deficiency is associated with an abnormally rapid rate of formation of the pigment (though there appears to be no reason for postulating this), or to assign significance to the finding of streptococcus viridans in the urine

Mode of action of ascorbic acid   The results of our in vitro experiments and our failure to demonstrate increased formation of bile pigments during treatment combine to show that removal of methæmoglobin by ascorbic acid is effected by reduction to hæmoglobin rather than by destruction of the abnormal pigment

The methæmoglobin level in the blood of these cases depends on the equilibrium established between the rate of formation and the rate of removal of the pigment   Gibson (14) has found that the reaction between methæmoglobin and ascorbic acid can be described by the bimolecular formula   The rate of reduction of methæmoglobin is thus proportional to the product of the concentrations of the two reactants *   The suggestion of a dynamic equilibrium is supported by the product [MHb] [ascorbic acid] being nearly the same before and after treatment   The curative effect of ascorbic acid would seem to consist in displacing this equilibrium by increasing the rate of reduction of methæmoglobin ,   the concentration of methæmoglobin in the blood would fall until the rate of reduction again became equal to the rate of formation at a new and lower methæmoglobin concentration While our cases have continued the treatment without intermission, thereby maintaining their blood methæmoglobin at a low level, it is probable that the therapy is in no sense curative   A reduction in ascorbic acid intake would be expected to lead to the accumulation of further quantities of methæmoglobin in the blood

The relative freedom from subjective symptoms enjoyed by our patients even before treatment, is worthy of comment, and is possibly to be explained by the initial polycythæmia and high total pigment concentration

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* The presence of glutathione in the RBC in amounts large as compared with the ascorbic acid will ensure that the latter is maintained in the reduced state   In vitro experiments with glutathione itself have shown that although it does reduce methæmoglobin, the rate of reduction is very slow compared with the rate of reduction due to ascorbic acid

The compensatory nature of this reaction is suggested by the fall in these values which took place during treatment (*see* Fig 1)

The success which has attended the use of ascorbic acid in these cases might well prompt its trial in other types of methæmoglobinæmia It is our opinion, however, based on the observed rate of recovery of our cases and the in vitro results of Gibson (14), that the effect of ascorbic acid in methæmoglobinæmia due to drugs would be so slight as to be negligible in comparison with the rate of reduction which might be expected in the presence of an intact corpuscular enzyme system

SUMMARY

1   An investigation is described of two brothers suffering from familial idiopathic methæmoglobinæmia

2   Blood changes in one of the brothers were followed during the course of successful treatment with ascorbic acid   The cyanosis was relieved and the methæmoglobin fell from 7 3 g /100 c c to 0 8 g /100 c c blood Continued treatment with ascorbic acid (200-300 mg per day) has kept the methæmoglobin at a low level for nearly two years

3   In vitro ascorbic acid reduced methæmoglobin in the patient's red cells to normal hæmoglobin

4   The enzyme systems in the erythrocytes of the two patients reduced methæmoglobin in presence of added glucose or lactate much more slowly than did those present in normal red cells

5   A summary is given of the main features of the few cases of idiopathic methæmoglobinæmia hitherto reported   The mode of action of ascorbic acid in relieving the symptoms, and the ætiology of the disease, are discussed

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EXPERIMENTAL LIMB ISCHÆMIA IN MAN WITH ESPECIAL REFERENCE TO THE ROLE OF ADENOSINE TRIPHOSPHATE

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OF recent years, the occlusion of the blood supply to one or more limbs has become a standard method of producing shock in animals (4, 42, 44) That interference with the blood supply to the limbs in man will produce serious effects is shown by cases of the " crush syndrome " following prolonged compression of the limbs by masonry, etc   Most of the experimental work on limb ischæmia in man has been concerned from a physiological point of view with the phenomena of reactive hyperæmia (23, 29) and from a pathological point of view with Volkman's contracture and traumatic arterial spasm (7)   Although it was pointed out by Cannon (18) that collapse frequently followed removal of tourniquets which had been left in place for a long time, little experimental work on this aspect of the effects of tourniquets in man appears to have been carried out

In the course of work on factors operative in the production of shock, Parsons and Phemister (35) observed the effects of a tourniquet applied to one leg   After a period of 15 min ischæmia they found that the systolic blood pressure fell from 106 to 102 mm Hg in two experiments while the pulse rate rose from 72 to 76 per min   Normal values had returned within 10 min and not much importance was attached to the finding Livingstone, McFetridge and Brunner (30) found that the application of a tourniquet did not produce any appreciable change in the blood pressure if the patient was fully anæsthetised, but if not, then a slight rise might occur   After removal of the tourniquet, 65 of 75 subjects showed a fall in blood pressure , in 31 of the 65 the pulse rate diminished and of the remaining cases, 10 showed cardiac acceleration   These results are difficult to evaluate as neither the method of application of the tourniquets, the duration of the limb ischæmia nor the extent of the changes in blood pressure or pulse rate

are given   Alam and Smirk (2) found that there was little change in the pulse rate during the first 10 min of arrest of the circulation to the resting leg of the unanæsthetised subject but after 10 min there was an appreciable increase in the rate in some subjects   Wilson and Roome (44) show a graph of the blood pressure changes in a man who had a tourniquet applied to one leg for two hours during an operation under anæsthesia   There was a marked fall in blood pressure on removal of the tourniquet, the blood pressure having been raised during the period of ischæmia   The blood pressure returned to normal after a few minutes to be followed by a secondary prolonged fall about 20 min later

Precise knowledge concerning the reactions which occur during and following the application of tourniquets in man would appear to be lacking In view of the importance attached to the use of tourniquets in war time, it seemed that a re-investigation of this subject was wanted   It was also desired to determine, if possible, whether or not adenosine triphosphate (ATP) played any part in these reactions as it had previously been shown by Green (26) and Bielschowsky and Green (12) to be capable of producing a shock-like state in animals

Methods

The investigations were carried out on 11 males whose ages ranged from 19 to 33 years   Seven of these were receiving a diet deficient in vitamin A but their reactions differed in no way from those receiving the vitamin

The subject lay comfortably at rest on a raised stretcher   Care was taken to avoid movement of the limbs in order to exclude the pulse accelerating and blood pressure-raising reflexes described by Alam and Smirk (1, 2)   The blood pressure, pulse rate, respiratory rate and buccal temperature were recorded at intervals throughout the period of observation which extended up to 2 hours after the release of the tourniquets   Subjective sensations experienced by the subject were also noted   The blood pressure determinations were made with a mercury sphygmomanometer, and the temperature with a mercury thermometer   Other observations were made in some cases and will be referred to in the text

When the subject had been lying for 15 to 20 min and the recordings showed that a steady state had been reached, tourniquets (rubber Esmarch's bandage) were applied to both legs so as to occlude the circulation to the legs as judged by the disappearance of pulsation in the dorsalis pedis arteries Two methods of application were used —(1) the tourniquet was simply bound round the mid-thigh   (2) the legs were elevated for a few minutes and the rubber bandages were then applied, binding from the foot upwards so as to render the limb, as far as possible, bloodless   The methods will be referred to as methods I and II   The tourniquets were kept in position for 25 min

Results

1   *Changes occurring during the period of ischœmia*   Similar changes occurred during the 25 min period of arrest of the circulation to the legs in both types of experiment and the results are therefore considered together They are summarized in Table I

TABLE I.

Observations before and at the end of a 25 min period of circulatory arrest to both legs

Subject	Method	Oral temp °F		Pulse rate		Syst B.P		Diast B P		Resp rate	
		A	B	A	B	A	B	A	B	A	B
B H.H	I	98 2	98 2	64	78	134	164	66	100	12	12
	II	98 4	96 8	72	92	118	152	60	76	14	18
C.L W	I	98 3	98 8	84	100	126	118	80	78	18	12
	II	98 6	98 1	96	92	124	134	76	88	16	20
E H D	I	98 9	99 0	72	72	116	124	58	74	16	20
	II			76	84	106	140	50	92	18	16
H G	I	97 7	97 8	64	68	136	148	78	98	12	12
	II	97 8	97 8	64	72	122	142	84	102	12	16
L T	I	97 4	97 7	64	84	126	150	74	96	16	16
	II	98 4	98 5	80	92	142	138	72	82	22	15
N W.P	I	98 4	97 2	72	68	118	129	72	100	12	12
	II	98 4	97 8	72	76	114	138	58	98	12	14
J.D	I	98 6	97 6	48	68	116	140	70	98	12	8
	I	97 2	97 7	52	68	114	134	64	86	16	20
	II	98 7	98 3	60	68	120	128	68	70	20	20
G D S	I	99 2	98 8	72	84	136	142	88	100	20	20
	I	98 0	98 3	76	84	124	140	80	86	16	16
	II	98 5	98 7	68	80	128	130	76	80	20	20
D W	I	99 1	99 2	64	72	116	124	78	60	16	16
H B	II	98 1	98 0	72	80	126	152	76	100	16	16

Method   I—simple application to the thighs

  II—application with preliminary binding of the legs from the foot upwards

The figures under A and B are the readings just before the application of the tourniquets and just prior to their release respectively

When the tourniquets were properly applied, the immediate pain was not excessive, it lasted for about five minutes and was greater with method II After this the legs became numb and there was little more than a dull ache until towards the end of the 25 min when the pain became somewhat intensified This increased pain was presumed to be due to the inability of the tourniquets to occlude the blood vessels of the femur so that blood was seeping into the limbs and was unable to leave owing to the more complete occlusion of the veins Although the pain of application was greater with method II, once that had passed off there appeared to be no difference between the two methods in this respect

Six of the subjects in 10 experiments performed on them, complained of feeling hot during the period of circulatory arrest The time of onset of this sensation varied from 3 to 24 min after the application In one subject (B H H) this was accompanied on both occasions by general sweating This was the only subjective sensation other than pain observed during the period of limb ischæmia

There was a slight rise in the buccal temperature in some subjects The figures in *Table I* do not bring out this point as the oral temperature was often falling just prior to the release of the tourniquets A rise in temperature occurred in 13 of 20 experiments, the average rise being 0 3°F (range 0 1 to 0 8°F) The peak of the rise was reached at around 15 min and usually preceded the sensation of heat which was sometimes accompanied by flushing of the skin Earlier experiments with shorter periods of occlusion showed that the extent of the rise was not proportional to the period of ischæmia There is naturally some doubt as to whether such a small rise in temperature is significant Its frequent occurrence, the subjective sensation of heat and the flushing of the skin do however suggest that in some subjects the body temperature rises during limb ischæmia

The most striking changes were in the cardiovascular system A rise in blood pressure occurred in every experiment (shown in all but one in Table I) It will be seen (Fig 1), that whilst the general level of the systolic blood pressure was higher than in the control period, the rise was characterised by two small peaks This was observed in all except three of the experiments, when only the second peak occurred The first peak was seen within a few minutes of the application of the tourniquets after which the systolic pressure fell, to rise again to a second peak shortly before their removal The increase in the diastolic pressure was relatively greater than that of the systolic with a consequent reduction in the pulse pressure during this period Acceleration of the pulse rate accompanied the rise in blood pressure in all but one experiment (N W P)

There were no constant changes in the respiratory rate Sometimes there was an increase in the depth of respiration which seemed to be associated with the degree of pain When the pain was not severe breathing tended to be shallower than normal, an observation also made previously (8)

2 *The immediate effects of release of the tourniquets* Immediately after release there was mild pain in the legs in all instances lasting about 3 min The pain was of similar intensity with both methods of application There were also other subjective and objective manifestations In general both the incidence and severity of the symptoms after method II were less than after method I A momentary sensation of faintness occurred in about half the tests and was more pronounced after method I , actual loss of consciousness was not observed Generalized tremors occurred and in one man (N W P), began before the removal of the tourniquets Using method I
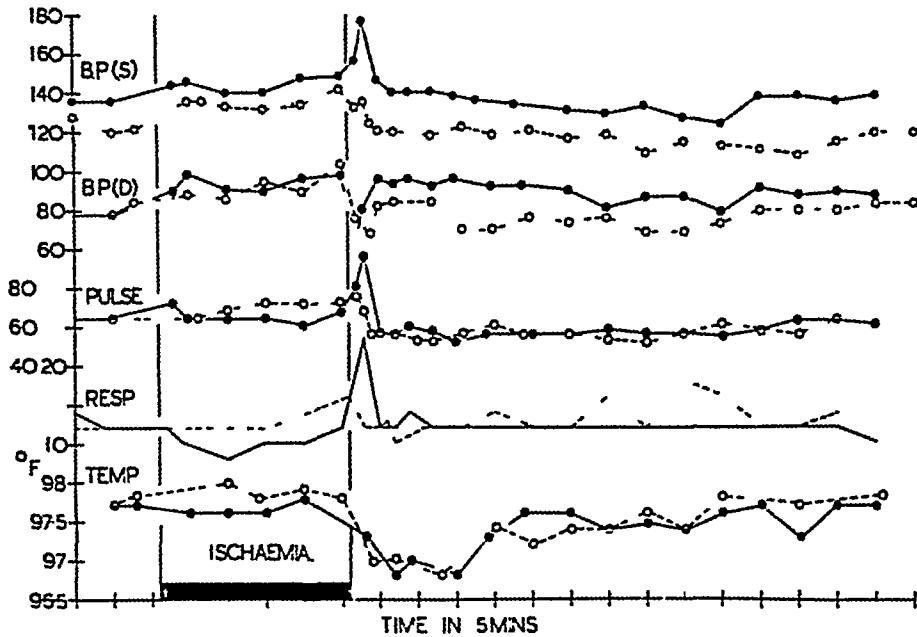
Fig 1 (Subject H G) Shows the changes in blood pressure pulse rate respiratory rate and oral temperature (°F) during and after arrest of the circulation to both legs for 25 min Method I continuous line Method II broken line

the average time of onset of the tremors, which occurred in 6 of 9 tests, was 3 min after release whereas with method II the onset was delayed till 7 min and then only occurred in 3 of 9 tests

One of the most striking changes following the removal of the tourniquets was a fall in the oral temperature , this occurred in 16 of 17 experiments The average fall was the same in both types of experiment, being 1 1°F (Group I, 0 6 to 1 7°F Group II, 0 6 to 2 5°F) The temperature was depressed for approximately the same time in both groups, averaging 25 min in I, and 22 min in II The fall usually started immediately after the removal of the tourniquets but in 6 instances it began

shortly before   The minimum temperature was reached sooner in Group II than in Group I, namely, in 6 min (3 to 12 min) compared with 9 min (6 to 22 min)

The cardiovascular changes immediately after release (see Fig 1), were affected by the method of applying the tourniquets   Thus with method I the systolic blood pressure rose further after removal in 5 experiments, fell in 3 and in one there was no alteration, with method II it fell in 6 and rose in 3 experiments   The degree of these changes was variable and could not be correlated with any other event occurring at this time   They were not long-lived, and in every subject the systolic blood pressure had returned to normal within about 10 min but its behaviour during that period differed in the two groups   In group II the systolic blood pressure returned gradually to normal, whereas in group I there was a series of fluctuations in the pressure, the primary tendency being to rise   The changes in the diastolic pressure were more uniform   In all subjects in group I it fell and in 6 experiments the fall was such that the second sound could not be detected   In group II the diastolic pressure fell in 6 experiments, rose in 2 and showed no change in one   The fall was not so great as in group I and in no case was the pressure unrecordable   After this primary fall the pressure rose over a period of 5 min in both groups to above the normal level, and after remaining at this level for 5 to 15 min gradually fell to its original value   It will be seen then that in general the pulse pressure was greatly increased in the first 5 min after release, and was followed by a fall to below the normal level   The primary change is much greater with method I than with method II

The changes in the pulse rate immediately following the restoration of the circulation in the legs were as follows   Group I —in 7 of 9 experiments there was a rise (average 17 per min), in the remaining two there was no immediate change   This lasted for 3 min after which the pulse rate gradually fell about 12 beats (range 4 to 28) per min below the original level   Group II —5 subjects showed a rise (aver 6 per min), 3 showed a fall (aver 11 per min) and one showed no change   With the second method of tourniquet application cardiac acceleration was much less evident immediately following release   After this phase however, brady-cardia of similar degree and duration followed in both groups   The period of bradycardia was very variable, and in some experiments the pulse had not reached its original rate after 2 hours

The effect on the respiratory rate also varied with the method of applying the tourniquets   With method I the respiratory rate increased immediately after release in the majority of experiments and dyspnœa was experienced by seven subjects   With method II it increased in only two instances and decreased in four, dyspnœa was experienced by 3 subjects   Not much additional information was gained from the tracings of the respiratory movements which were made in 11 experiments except that, coincident with the increase in rate, an increase in respiratory amplitude was noted   Restoration of the circulation to the resting ischæmic limbs

would appear to produce a temporary increase in pulmonary ventilation
In Fig 1 representative findings are shown which illustrate that the changes
in blood pressure pulse and respiration (like the subjective phenomena)
are less after method II than after method I

   3   *The delayed effects after release of the tourniquets*   There were no
significant differences between the two methods of tourniquet application
in the changes which occurred between ¼ and 2 hours after restoration of the
circulation to the limbs   The results will therefore be treated as a whole

   In most experiments the subject felt completely normal during this
period   Borborygmi were heard in 7 of 18 experiments about half an hour
after the release of the tourniquets and in one subject were accompanied
by colic

   There were no changes in either the rate or depth of respiration but
there were changes in the cardiovascular system   In 10 experiments there
was a fall in the systolic pressure (average 10 mm , range 2 to 18) which in 8
was accompanied by a decrease in the pulse pressure (aver 14 mm , range
4 to 30), whilst in two other experiments there was a reduction in the pulse
pressure without any significant change in the systolic pressure   The fall
commenced 3 to 53 min after the removal of the tourniquets and lasted
for an average of 25 min (5 to 47 min)   The type of fluctuation produced
is shown in Fig 1   As noted before, the pulse rate during this period was
frequently below the initial level

Discussion and further observations

   The most important changes during a 25 min period of ischæmia of
both lower limbs are those in the cardiovascular system   One possible
explanation of the rise in blood pressure is that it is a mechanical effect
due to the reduction in the vascular bed   Bearing on this is the work of
Barcroft (5) who showed that occlusion of the thoracic aorta of the dog
produces an increase in blood pressure and cardiac output, especially after
section of the vagi   With Formijne (6) he demonstrated that this effect
was independent of the nervous system and was a mechanical one   If such
were the explanation of our results then one would expect that the rise in
blood pressure would be greater when the limbs were made bloodless before
occlusion of the circulation, than when part of the circulating volume was
cut off in the limbs   This was not so   Furthermore, if the effect is
mechanical it should occur under deep anæsthesia   This was tested in
six patients in whom tourniquets were applied after the induction of
anæsthesia prior to operation (meniscectomy, etc)   In deep anæsthesia
produced by a chloroform-ether mixture followed by ether it was found,
in agreement with Livingstone, McFetridge and Brunner (30) that no rise
in blood pressure occurred   It appears probable, therefore, that the rise is
reflex in origin   Alam and Smirk (1, 2) have shown the presence of blood
pressure-raising and pulse accelerating reflexes arising in the muscles of
ischæmic limbs during exercise   They consider that the exciting agents

with or without exercise are muscle metabolites acting upon afferent nerve endings, and that pain stimuli are not involved (3) Whilst agreeing that the changes are probably reflex in origin, we are not so certain of the nature of the stimulus In the present work, the blood pressure and pulse curves showed two peaks which were coincident with the time of greatest pain, namely at the time of application and just prior to release This view is supported by the observation of Eichna and Wilkins (23) that only when pain was felt during the period of ischaemia was there a rise in the blood pressure

With regard to those changes occurring immediately after release of the tourniquets it is necessary to elucidate both the changes themselves and the differences between them with the two methods of application

The most constant effect was the fall in oral temperature and a possible explanation is the loss of heat through the hyperaemic areas The skin temperature of one leg, above and below the site of the tourniquet, was followed with a thermocouple in three experiments and the peak of the increase seen during the period of reactive hyperaemia was found to coincide with the point of maximum depression of the oral temperature This explanation is supported by the fact that neither the degree of the reactive hyperaemia nor the fall in oral temperature was greatly affected by the way in which the tourniquets were applied There were however minor differences in the latter, and these, coupled with the fact that in 6 of 17 experiments the decline began before the release of the tourniquets, suggest that although hyperaemia of the limbs is probably the main factor, it may not be the only one The sensation of coldness was obviously associated with the fall in temperature as were probably the muscular tremors which occurred

The explanation of the increased pulmonary ventilation is to be found, in part, in the work of Barman, Moreira and Consolazio (8) They occluded for short periods the circulation to the legs of subjects who were walking on a treadmill On restoring the circulation there was an increase in the pulmonary ventilation, which was accompanied by a marked increase in the blood lactate Part of the increase in oxygen intake was ascribed to the removal of this lactate Also, Billings and Maegraith (14) have shown that the reaction of the blood leaving an ischaemic limb is more acid than normal and this offers an additional reason for the increase in pulmonary ventilation The experiments described here are not directly comparable but it is probable that a similar mechanism is at work This would explain the differences seen following the two methods of tourniquet application for with method II there was a short latent period before the onset of deeper breathing which was not present following method I In method I, blood of lower pH than normal which has been pent up in the legs would be suddenly liberated on release of the pressure, whereas in method II, a short time must elapse before the constitution of the normal blood entering the limb is changed However, as will be seen later, these may not be the only factors involved

As to the cardiovascular changes, two possible explanations are suggested (1) they are secondary to the respiratory changes and (2) they are due to the liberation of substances which have accumulated in the limbs during the period of ischæmia   Owing to the differences seen following the two methods of application the changes cannot be explained by a neurogenic theory nor can they be secondary to the fall in body temperature   That they are secondary to the respiratory changes seems unlikely for the cardiovascular effects of hyperpnœa are very variable (15), and they have been shown (19, 32 40 43) to be minimal when the subject is recumbent, as in our experiments   Nor was the degree of hyperventilation sufficient to produce the observed changes in blood pressure and pulse   It would seem more likely that the cardiovascular effects were due to the release of metabolites into the blood

TABLE II

Substance	Species	Author
Adenosine containing substances	unknown	Zipf (13) Quoted Drury (21)
	rabbit	Billing and Macgraith (14)
	rabbit	Stoner and Green (11)
Histamine	dog	Barsoum and Gaddum (10)
	man	Barsoum and Smirk (11)
	rabbit	Billing and Macgraith (14)
		Disputed by
	man	Marcou Comsa and Chirireano (31)
	man	Kwiatlowski (28)
	rabbit	do
Potassium	cat	Fenn Wilde Boak and Koenemann (24)
	man	Re cell (37)
Lactates	man	Barman Moreir and Com olazio (6)
Myohæmoglobin	man	Bywaters et al (16)
Inorganic phosphates	dog	Duncan (20)
		Mylon Winternitz and de Suto Nagy (34)
	rabbit	Stoner and Green (41)
Creatine	man	Bywaters (17)

Substances which have been shown to be liberated into the blood stream from ischæmic limbs with a summary of the literature

The nature of substances liberated into the blood stream from ischæmic limbs has long been a matter of physiological and pathological interest A list of the substances which have been found to be so liberated is shown in Table II   In the main these substances are derived from the muscles of the ischæmic part

Lewis and Grant (29) considered that histamine or some histamine-like body might play a part in reactive hyperæmia   In support of this, Barsoum

and Gaddum (10) and others (11, 14) found an increase in histamine in the blood leaving the ischæmic limb The point has been re-investigated by Kwiatkowski (28) who concluded that the blood histamine was not significantly increased and that histamine was not concerned in the general disturbance following reactive hyperæmia Our findings support the second of these conclusions for flushing of the face and headache (symptoms which follow the administration of very small amounts of histamine, (11)) were each only observed on one occasion Nor was it possible, by the intravenous injection of histamine in varying amounts to produce changes similar to those following removal of the tourniquets Rewell (37) has recently demonstrated a rise in serum potassium in a series of experiments with tourniquets rather similar to those described here The increases reported by him are small and it is doubtful if they play any part in the production of symptoms, especially in view of the work of Winkler and Hoff (45) who found that potassium intoxication played little or no part in the death of dogs dying as a result of limb ischæmia

As already pointed out one of our main interests in these experiments has been to determine whether or not adenosine triphosphate (ATP) had any role in the causation of the changes described Both Fleisch and Weger (25) and McDowall (33) have suggested that ATP may play a causative role in reactive hyperæmia In agreement with Zipf (47) and Billings and Maegraith (14) we have previously shown (41) that limb ischæmia in the rabbit is accompanied by an increase in the adenosine equivalent of the blood It is therefore important to decide whether limb ischæmia is accompanied by such a rise in man

Adenosine estimates Accordingly observations were made on six normal male subjects whose ages ranged between 19 and 21 After the subject had been lying at rest for 10 min blood was removed from the antecubital vein without obstruction at 0, 30, 34 and 50 min This period was the control period, the venepunctures being similarly spaced to those in the experimental period which followed At the 55th min tourniquets were applied to both legs (method I) and kept there until the 85th min Further blood specimens were taken at 82, 87 and 100 min The blood adenosine was extracted using the method of Barsoum and Gaddum (9) modified for 2 0 c c of blood Heparin was the anticoagulant and the extraction was commenced immediately after withdrawal The hæmoglobin concentration (Haldane) of each specimen was determined. The extracts were assayed against adenosine (B D H) using the modified guinea-pig auricle preparation (41, 22) The accuracy of this method was found to be ± 10 per cent Each extract was assayed on at least two separate preparations and each value given for the adenosine equivalent is an average of the two adjusted for any alteration in the blood Hb by correction to a standard level of 100 per cent. No attempt was made to fractionate the blood into corpuscles and plasma, and all the values shown for the adenosine

equivalents refer to whole blood. It might be reasoned that since the increase in the adenosine equivalent which could be expected on theoretical grounds is small, it would have been more profitable to have examined the plasma in which the normal concentration of adenosine containing substances is low. This was not done because it has been shown by Phemister and Handy (36) that very slight trauma to blood after withdrawal results in its acquiring vasodilator properties due to the liberation of adenylic acid or a compound containing it (Zipf (46)). Furthermore, Barsoum and Gaddum (9) have shown that slight degrees of trauma to the blood leads to alterations in the adenosine equivalent of the plasma. It would therefore be difficult to decide on the significance of any small increase in the adenosine equivalent of the plasma.

TABLE III

Effect of a 30 min period of circulatory arrest to both legs on the adenosine equivalent of whole venous blood. Tourniquets applied from 55th to 85th min of the experiment

Subject	Time when specimen taken (min.)						
	0	30	34	50	82	87	100
	Adenosine equivalent (μg/ml)						
J.D	130	150	150	200	170	280	270
G D S	110	130	130	110	110	150	120
G D S	—	—	—	130	—	150	150
J A C	210	210	220	190	220	230	200
G R W	140	130	140	130	140	170	140
A R C	140	150	—	150	160	170	150
A M	130	130	120	130	130	200	170

It will be seen from Table III that the adenosine equivalent of the whole blood 2 min. after release of the tourniquets was greater than at any other time. The average increase was 33 per cent (range 11 to 79 per cent) and in 5 experiments it had reached or was approaching the normal level within 15 min. This increase has been examined statistically and is significant. Limb ischæmia in man is followed therefore by an increase in the adenosine equivalent of the blood. The exact nature of the substance which causes this increase is not known but there are theoretical reasons for identifying it with adenosine triphosphate (Stoner and Green (41)) and more recent animal experiments tend to substantiate this view.

To allot a part to ATP in the production of the general effects of reactive hyperæmia, in addition to demonstrating that limb ischæmia increases the blood concentration, it must be shown that the intravenous injection of

small amounts of ATP reproduces some at least of these effects, that those subjects who have become tolerant to injections of ATP react less after limb ischæmia, and that sensitivity to limb ischæmia varies in the same way as sensitivity to ATP

Adenosine triphosphate injections The effect of adenosine and allied compounds in animals has been reviewed by Drury (21) The action of ATP in man has not been directly investigated but that of adenosine and adenylic acid has been studied from a few aspects and the relevant references will be found in the above review Our investigation of the action of ATP was made in a series of 7 male subjects whose ages ranged from 19 to 33 years Both the sodium and the magnesium salts were used, these being prepared from the barium salt The doses ranged from 5 to 40 mg In an experiment lasting 3 hours where 50 to 80 mg were given in divided doses no cumulative effect was observed In man as in animals the magnesium salt is the more active and gives a closer approximation to the picture seen after release of the tourniquets This accords with our view that the magnesium salt is the biologically active one (13)

Sensitivity to intravenous ATP varies Four subjects received both intravenous injections of ATP and underwent tourniquet experiments Their order of sensitivity was the same to both procedures, the correlation between the degrees of bradycardia being particularly clear

With successive injections of increasing doses of ATP the first effect observed was upon respiration An increase in both rate and depth occurred, usually accompanied by the sensation of dyspnœa The effect was seen after doses as small as 0 106 mg /kg body wt and was proportional to the dosage With the larger doses (0 3 to 0 4 mg /kg body wt) the disturbance persisted up to 8 min

The cardiovascular system was next affected, the main change occurring in the pulse rate With the smaller doses of ATP (up to 0 3 mg /kg body wt) the main effect was tachycardia which usually persisted for a minute during which the pulse rate might be trebled At the end of the minute the pulse rate returned abruptly to normal where it remained after the smaller doses With slightly larger doses the depressant action of ATP on the heart became more evident The primary rise in pulse rate was less or absent and was always followed by a period of bradycardia With larger doses the depressant action was even more evident This transition from stimulation to depression was well shown by subject E H D Here a dose of 10 mgm NaATP (0 152 mg /kg body wt) increased the heart rate by 32 beats per min with a return to normal within 4 min Double this dose produced no immediate change but after 3 min slowing of the heart occurred, lasting for 6 min (maximum depression 14 beats a min), 30 mgm produced a profound slowing of the pulse which lasted for one minute after which the pulse rate gradually rose to normal over a period of 10 minutes This was the only experiment in which heart block might be suspected ATP appears

to have less action on the conducting tissue of the human heart than adenosine and muscle adenylic acid (27, 38, 39)   The changes in the pulse rate after a moderate intravenous dose of ATP are illustrated in Fig 2   These changes, and in particular the period of bradycardia are much like those observed after release of limb tourniquets
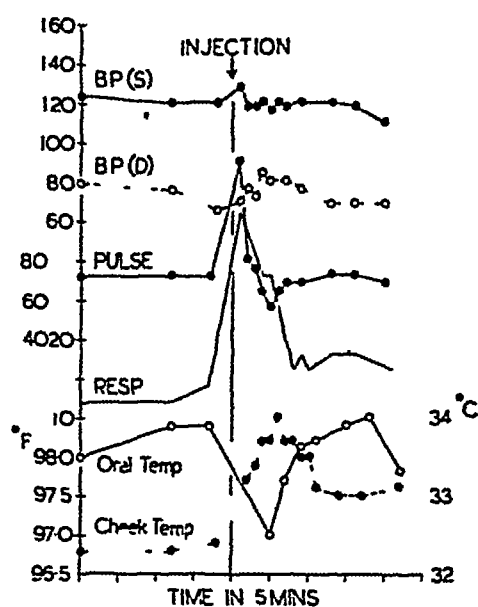

Fig 2   (Subject G.D.S)   Shows the changes in blood pressure, pulse rate respiratory rate and oral and cheek temperatures after the intravenous injection of 20 mg magnesium adenosine triphosphate (0 316 mg /kg body weight)

The changes occurring in the systolic blood pressure reflected those in the pulse rate   The systolic pressure was raised somewhat during the primary cardiac acceleration to be followed by a slight fall when the heart rate was depressed   The type of change is shown in Fig 2 where it will be seen that changes also occur in the diastolic pressure   During the phase of cardiac acceleration it usually fell sometimes so far as to be unrecordable   After this it returned to its original level but tended to rise during the period of bradycardia, thus producing a fall in pulse pressure

We have also investigated the effect of ATP on the peripheral vessels The central artery of the retina was observed in two subjects during and following the intravenous injection of ATP   Although the doses used were maximal for the individuals concerned no alteration in calibre was observed In three subjects ATP was injected into the brachial artery in doses of 2 to 4 mg   The result was a patchy bright erythema accompanied by tingling pains in the forearm and a rise in the temperature of the affected skin   The changes appeared after a latent period of 30 to 45 secs   and

lasted for 3 to 6 min   We interpreted this as due to arteriolar dilatation The reaction of the capillaries was observed in two ways   The capillaries at the corneo-scleral margin were observed with a slit lamp microscope during and following the intravenous injection of ATP   No change was observed in the calibre of the vessels   Intradermal injection into the skin of the forearm of 2 0 mg   dissolved in 0 5 c c   saline produced a flare of moderate intensity around the area of the injection which itself remained pale   When this was followed, 10 min   later, by the intravenous injection of 10 c c   Evans' Blue (T 1824) the centre became stained with the dye The effect on the capillaries would therefore appear to be mainly one of an increase in permeability   There was also evidence of local fluid loss when doses of 25 to 50 mg   were given subcutaneously   These doses had no general effects but led to severe pain with local swelling and induration which lasted for about 1 hour   In man, therefore, ATP would appear to exert its main peripheral effect through the arterioles   This would account for the changes in the skin temperature   It will be seen (Fig 2) that the intravenous injection of ATP is followed by a rise in the temperature of the cheek as measured with a thermocouple   This was a constant finding, the average maximum rise being 1 0 to 1 5°C   irrespective of the dose employed   The increased temperature lasted for an average of 10 min and in some cases was accompanied by flushing of the face   This was short-lived and sometimes preceded by pallor   An increase also occurred in the

TABLE IV

Details of a tolerance experiment on G S and J D   Adenosine triphosphate given intravenously at the same time each day   No appreciable variation in the body weight of either subject during the course of the experiment

Day of Exp	Dose Mg ATP (mgm)		Fall in oral temp (°F)		Rise in cheek temp (°C)		Immediate rise — Syst B P (mm Hg)		Pulse rate		Period of resp effects (min)	
	G S	J D	G S	J D	G S	J D	G S	J D	G S	J D	G S	J D
1	20	25	0 4	—	1 8	1 0	10	12	64	76	7	7
2	20	25	1 4	0 3	1 3	1 3	8	2	58	68	7	5
3	20	25	0 8	0 2	2 0	0 6	16	2	62	52	7	3
4	20	25	0 4	0 4	1 7	1 0	6	6	52	36	5	7
5	20	30	0 9	0 6	2 6	1 3	−4	8	46	20	2	3
6	25	35	0 7	0 5	1 5	0 5	−4	2	44	22	3	5
7	25	40	1 4	0 8	1 5	0 7	4	−2	16	28	4	5
8	30	50	1 1	0 5	2 5	1 0	−8	2	16	22	3	2
	35	60	1 1	0 1	2 5	2 0	4	7	76	−4	8	5

skin temperature elsewhere (forearm) but was not so constant nor did it last so long   Loss of skin heat would seem sufficient therefore to explain the slight fall in oral temperature seen after the larger doses (Fig 2)

Tolerance to ATP was induced in two subjects and it will be seen from Table IV that a certain amount of tolerance was developed to the cardiac and respiratory effects of the injections   The last day's experiment was designed to see how much ATP had to be given to equal the effects produced by the original dose   It was impossible to determine how long the tolerance so acquired lasted, but in one subject (J D), it was still present a week after the end of the experiment

Tourniquet experiments (method I) were performed before and at the end of this period   The changes occurring during the period of ischæmia were unaltered but, immediately following the removal of the tourniquets, the subjective phenomena were somewhat less in the second experiment in both men   The fall in body temperature occurred as before and to the same degree, which, together with the fact that the degree of hyperæmia was unaltered, favours the view that it is due to loss of surface heat   In contrast, the disturbances in respiration, both in rate and depth, were less severe and lasted a shorter time in the second experiment   The alterations in pulse rate were also less pronounced , in one subject the tachycardia and rise in systolic blood pressure previously observed did not occur, and in both the ensuing period of bradycardia was distinctly shortened

It would seem therefore that the effects of intravenous ATP and those following limb ischæmia in man have these features in common —dyspnœa with increase in rate and depth of respirations, initial tachycardia followed by prolonged bradycardia and a pronounced increase in pulse pressure followed by a fall to below normal   In addition the similarity in the subjective sensations was commented on by all subjects   Other common features of which the significance is more doubtful, are the fall in body temperature and evidence of peripheral arteriolar dilatation   These facts, together with the rise in the adenosine equivalent of the blood following limb ischæmia, would appear to make a prima facie case for alloting some part to ATP or adenosine compounds in the reactions following limb ischæmia ,   the sensitivity and tolerance experiments give further support to this view   It is most unlikely that ATP is the sole responsible agent, for there is evidence that many other tissue metabolites gain access to the blood in increased amounts   The delayed onset of effects after release when the tourniquets were applied so as to reduce the blood volume of the limbs is in favour of tissue metabolites being important causative factors and, amongst these, ATP may well have the predominant role

The cause of the delayed effects after a period of limb ischæmia is not so clear   The significance of the borborygmi is doubtful though it may be noted that they occasionally occurred after ATP injections at corresponding times   The time relationships of the cardiovascular changes are similar to those shown in the graph of Wilson and Roome (44)   Adenosine-containing

substances would not appear to be directly involved, for the adenosine equivalent of the blood had returned to normal by this time, and the effects appeared in the ATP tolerant subjects   A possible explanation is that gradual local fluid loss into the ischæmic limbs is responsible for the fall in blood pressure   Evidence of this was sought along two lines   The hæmoglobin concentration was examined in eight subjects but no significant change was found   Attempts were also made to record the volume of the limbs   The girth of the calf and thigh was measured at frequent intervals during each experiment but again there was no significant change   A limb plethysmograph (in five experiments) showed a transient increase in limb volume immediately after the release of the tourniquets but no increase at later periods   No explanation of this secondary fall in blood pressure can yet be offered but a study of the effects of longer periods of ischæmia might reveal the cause

SUMMARY

1   The general bodily reactions during and following a 25 minute period of ischæmia of both lower limbs in 11 male subjects are fully described and their significance discussed

2   The effect of 30 minutes ischæmia of both legs on the adenosine equivalent of whole blood has been determined in 6 subjects and a small but significant increase was found

3   The pharmacological action of adenosine triphosphate on the respiratory and cardiovascular systems in man has been determined in 7 subjects and the results fully reported

4   The combined results of these three lines of investigation indicate that adenosine triphosphate may play a part in the general effects which occur immediately following the restoration of the circulation

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DEVELOPMENT AND COURSE OF EXOPHTHALMOS AND OPHTHALMOPLEGIA IN GRAVES' DISEASE WITH SPECIAL REFERENCE TO THE EFFECT OF THYROIDECTOMY

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IN this paper an attempt is made to trace the natural history of exophthalmos, ophthalmoplegia and related signs of Graves' disease   Serial measurements of the prominence and range of elevation of the eye have been plotted and the curves correlated with changes in the resistance of the eye to light pressure and, the state of the conjunctiva   To be adequate, observations must be continued for months or years and clearly this is not practicable when fully developed Graves' disease is present ‡   We have made the relevant observations on patients suffering from the ophthalmic form

No detailed information concerning the development and course of exophthalmos has been available hitherto and misleading statements are common   Thus it has been asserted that exophthalmos may develop abruptly, even over-night, and that its extent may vary greatly from day to day   Evidence from measurements provides no support for such claims

The occurrence of the ophthalmic form demonstrates that in such cases at least, the ocular changes do not depend on hyperthyroidism   It is also shown in this paper that in thyrotoxicosis, exophthalmos and ophthalmoplegia are little affected by thyroidectomy   These observations emphasize the fundamental importance of the ocular changes in studies of the nature and pathogenesis of Graves' disease

NATURAL HISTORY OF OCULAR CHANGES

Method   In attempting to observe variations in exophthalmos the difficulty is at once encountered that lid retraction, with its consequent widening of the palpebral fissure, creates the illusion of exophthalmos, and

variations in lid retraction, which may be rapid and conspicuous, that of corresponding fluctuations in prominence of the eye   Failure to appreciate this difficulty may account for misleading statements concerning exophthalmos   Confirmation of variations by measurement is highly desirable, if not essential   Measurements of ocular prominence given here have been made with Hertel's exophthalmometer, modified to give greater accuracy, by the inclusion of a central forehead stop   The range of elevation has been measured with the vertometer and has been taken as the criterion of eye movements (8)
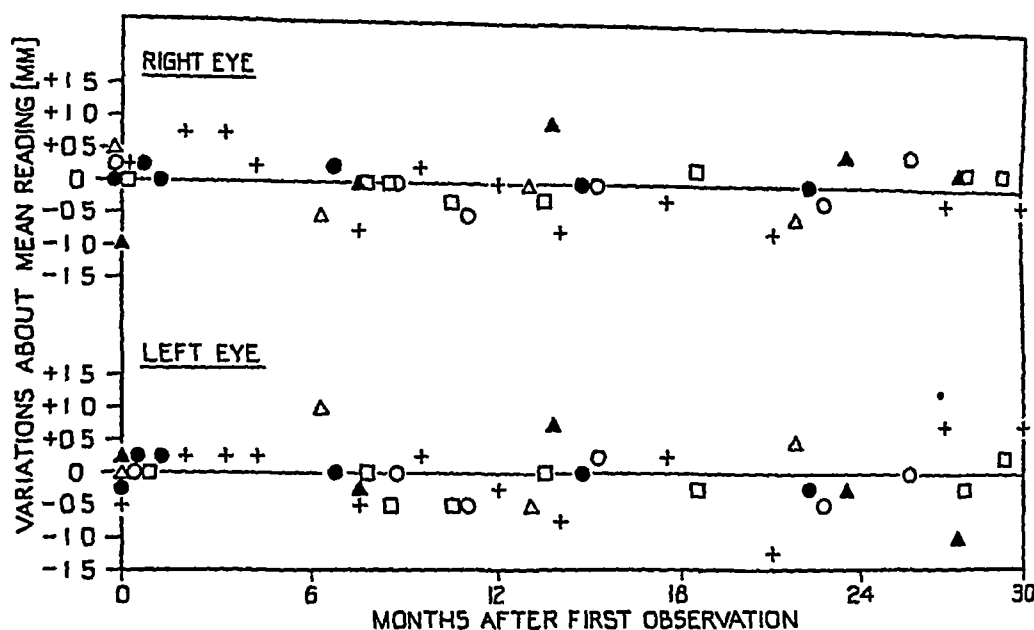
Fig 1   Static phase of exophthalmos   Mean values for readings of right and left eyes are represented by horizontal lines, the deviations of individual readings from the means are shown   All the eyes were known to be proptosed by more than 3 mm

				Mean Hertel reading,	
O	First reading	1 year	after thyroidectomy		R 26 25 , L 25 75 mm
●	„ „	5 months	„ „	„ „ „	R 21 25 , L 20 5 mm
□	„ „	3½ years	„ „	„ „ „	R 29 5 , L 30 0 mm
+	„ „	9 years	„ „	„ „ „	R 26 0 , L 26 0 mm
△	Ophthalmic form, onset	1 year previously		„ „ „	R 21 5 , L 21 75 mm
▲	„ „ „	3 years „		„ „ „	R 30 5 , L 30 25 mm

Serial measurements of exophthalmos and elevation have been made on 12 patients in whom change was clearly occurring , 4, Cases 7 and 16-18, had the ophthalmic form of Graves' disease, the remaining 8 also had goitre and hyperthyroidism   Of the latter, 1, Case 11, with mild hyperthyroidism and slight eye signs remained untreated, hyperthyroidism subsiding spontaneously after 1 year , the other 7 were treated by thyroidectomy soon after coming under observation *   Before operation, ocular changes

* Within 2 weeks of the initial readings given in Table I unless otherwise stated in that table

were pronounced in 5, Cases 10 and 12-15, slight in 2, Cases 8 and 9 All showed major trends afterwards Details of the readings are given in Fig 2 and Table I

Patients with Graves' disease do not usually come under close observation while the eye is protruding, most present later, when exophthalmos is established and stationary The 6 chosen to illustrate
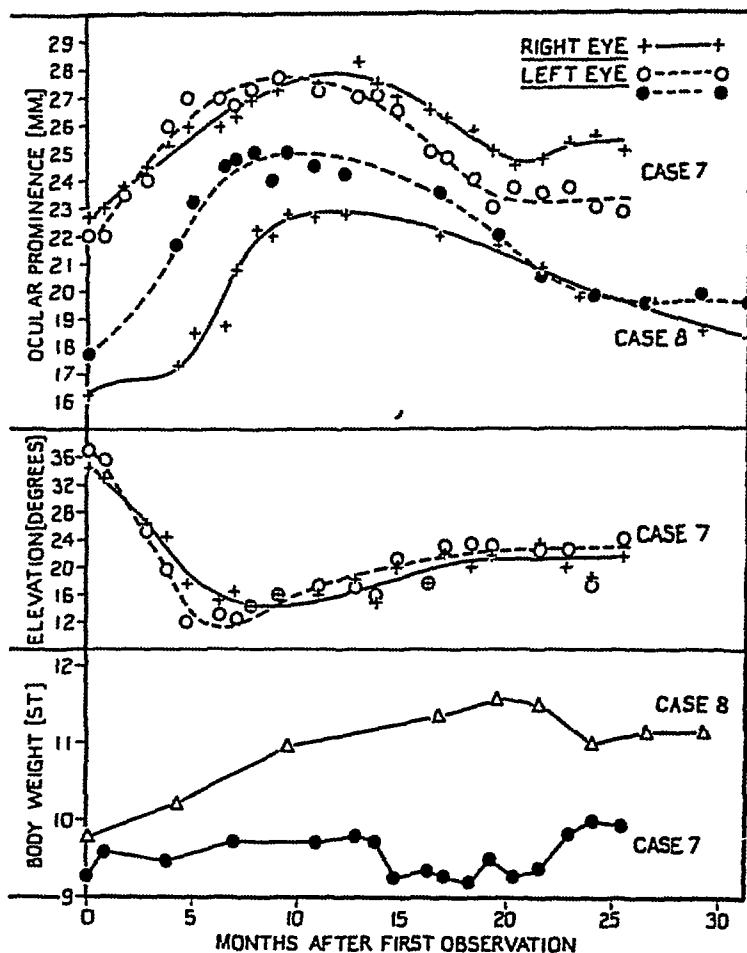
Fig 2 Case 7, ophthalmic form , Case 8 severe thyrotoxicosis with slight eye signs treated by thyroidectomy one week after first measurement Dynamic phase of ocular changes showing stages of ingravescence and remission The eye movements in Case 8 are detailed in a previous paper (8) Note the strong correlation between exophthalmos and ophthalmo plegia during ingravescence but maximum recovery of the latter precedes that of former Case 8 gained weight while the eyes receded In Case 7 pregnancy supervened during remission (after 17 months observation) this may have been a factor in the terminal gain in weight and protrusion of right eye

TABLE I   *Serial readings of elevation and*

Case No	Measurement at first observation			Measurement at intervals													
				1	2	3	4	5	6	7	8	9	10	11	12	13	14
9	Elevation (degrees)	R	30	—	—	36	—	—	—	36 5	—	—	29	26 5	26	26 5	24
		L	33	—	—	35 5	—	—	—	33 5	—	—	31	25 5	22 5	27 5	25
	Hertel reading (mm)	R	17 0	—	—	18 75	—	—	—	18 25	—	—	21 0	21 25	21 5	21 25	21 5
		L	16 0	—	—	18 0	—	—	—	17 25	—	—	18 0	18 75	18 75	18 75	19 5
10	Elevation (degrees)	R	36	—	—	33	26 5	25 5	—	19	24	21 5	27	22 5	23	26 5	21 5
		L	33	—	—	33 5	28 5	27 5	—	26	27	28	31	27	31	31 5	30
	Hertel reading (mm)	R	23 5	23 75	—	24 75	24 75	25 0	—	26 25	26 75	27	27 5	27 25	26 75	26 75	28
		L	24	24 75	—	24 25	23 75	23 75	—	23 5	23 75	23 75	23 25	23 25	23	23 25	23 25
11	Elevation (degrees)	R	38 5	36 5	34	—	36 5	31	—	33 5	—	30	34 5	—	30	30	—
		L	39 5	38	34	—	35 5	27	—	30	—	30	33	—	29	29	—
	Hertel reading (mm)	R	18 5	19	19	—	19	19 5	—	20	—	20	19 75	—	20	20 25	—
		L	18 5	19	19	—	19	19 5	—	19 75	—	19 75	20	—	20	20	—
	Weight (stone, pounds)			9 6½	9 4	9 2½	—	8 10	8 9	—	8 8	—	—	8 10	—	8 13	9 0	—
12	Elevation (degrees)	R	33	—	28 5	29 5	31 5	—	22 5	—	22	19 5	25	—	26 5	20 5	24
		L	30	—	25	30 5	29 5	—	22 0	—	20 5	24	24	—	24 5	22	22
	Hertel reading (mm)	R	25	—	27 75	27 0	28	—	28 75	—	28 5	28 75	28 75	—	29 25	29 75	30
		L	24 25	—	26 5	20 25	27	—	27 0	—	27	27	26 75	—	27 75	27 5	28
13	Elevation (degrees)	R	34 5	—	26 5	20 5	21 5	—	—	12	9 5	—	15	—	—	—	13
		L	12 5	—	13 5	10 5	12	—	—	16	17 5	—	20	—	—	—	16 5
	Hertel reading (mm)	R	25	—	26 75	27	27 5	—	—	28	28 25	—	27 5	—	—	—	27 5
		L	26 25	—	27 25	27 25	27 75	—	—	26 75	26 75	—	27	—	—	—	26 75

prominence of the eye in Graves' disease

Remarks

afterwards (months)														Remarks
15	16	17	18	19	20	21	22	23	24	25	26	27	28	
									10 5	—	8	14		Subsequent increase in Hertel readings to steady R 25 25 I 22 0 mm at 30 33 months when readings were discontinued Peak of exophthalmos may have been reached Elevation steady at R 15° L 11° during last 3 months Onset of progressive orbital changes 7 10 months after thyroidectomy
			—	20	21	—	11 5	4 5	8	0	5	—	5	8
23	—	—	20	17	—	12 5	8 5	7	0	5	—	24 0	24 25
21	—	—	22 5	22 75	—	23 0	23 75 24 25 24 75 24 75	—	21 75 22 0				
21 25	—	—	20 5	21 0	—	20 75 21 25 21 5	22 25 22 0	—	—	—	—		Thyroidectomy done 6 weeks after first reading * Progress of changes in right orbit concomitant with remission of those in left On the right exophthalmos became static at its maximum level Imperfect correlation between exophthalmos and ophthalmoplegia on left
19 25	—	—	—	—	—	—	25	—	—	—	—	
—	22 5	27	—	—	—	—	35	—	—	—	—	
—	28	33	—	—	—	—	27 5	—	—	—	—	
—	27 5	26 75	—	—	—	—	23 0	—	—	—	—	
—	23	23	—	—	—	—	—	34	—	—	—		Thyrotoxicosis goitre and systemic manifestations remitted spontaneously one year after first observation Shows slight persistent exophthalmos and ophthalmoplegia Weight was lost during development of exophthalmos
		—	28 5	29	32	29	32	—	35	—	—	
28	28	—	29 5	27 5	28	28	30	—	20 25	—	—	
29	28	—	20 25 20 5	20 25 20 25 20	—	20 0	—	—				
20	20 5	20 25	—				19 75	—	20 0	—	—	
20 5	20	20 25	—	20	19 5 19 75 19	19 75	—	0 0	—	—	—	
8 13	8 13	9 1	—	9 0	0 1	9 3	8 13 8 12	—	—	—	—		Orbital changes were already marked before thyroidectomy and progressed appreciably afterwards Remittent phase incompletely observed
			—	27	27 5	—	—	—	—	—	—	
21 5	—	26	21	—	30	27 5	—	—	—	—	—	
23	—	25	22	—	28 75 28 5	—	—	—	—	—	—	
29 75	—	29 75 29 25	—	27 25 27 0	—	—	—	—	—	—		
27 5	—	28	27 5	—	—	—	—	20	—	22	—		Orbital changes were pronounced before thyroidectomy and continued to develop afterwards Remission ill-defined with consequent, permanent, severe exophthalmos and ophthalmoplegia
		—	22 5	—	18	—	21 5	—	18 5	—	22	
—		—	24	—	23	—	19	—	27 5	—	26 75	—
—		—	26 5	—	26 75	—	27 5	—	25 5	—	26	—
—		—	26 25	—	25 75	—	26	—				

* Thyroidectomy was done within 2 weeks of first observation unless otherwise stated

TABLE I *Serial readings of elevation and*

Case No	Measurement at first observation		First obs	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	Elevation (degrees)	R	0	—	8.5	17.5	16.5	19	20.5	—	21.5	32	—	27.5	27	—	29
		L	18.5	—	18.5	25.5	23.5	25	24	—	27	35	—	29	26	—	27.5
14	Hertel reading (mm)	R	27	—	26.5	26	25	26	25 26	26.5	—	26.5	25.75	—	25 25	24 25	—	24
		L	24.5	—	25	24.75	24.75	24.5	25 25	—	24.5	24 25	—	24	24	—	23.75
	Weight (stone, pounds)		9 0	—	9 7	9 13	10 4	—	—	—	10 0	10 1	—	9 13	—	—	—
	Elevation (degrees)	R	36	—	—	26	28.5	26.5	24	—	22.5	24	—	20	—	—	26
		L	34.5	—	—	20.5	26.5	23.5	24	—	21	25	—	19.5	—	—	26
15	Hertel reading (mm)	R	21.25	—	—	24.5	24	23.5	23.75	—	25	24.75	—	24	—	—	23.25
		L	21.5	—	—	23.5	23.75	23.25	23.75	—	24.75	24	—	23.25	—	—	22.75
	Elevation (degrees)	R	7.5	—	8	—	0	3	4.5	9.5	9	—	—	—	18.5	18.5	19.5
		L	17	—	19.5	—	22.5	22.5	22.5	25	22	—	—	—	23.5	27	22.5
16	Hertel reading (mm)	R	26.25	—	27.5	—	27.25	28	27.5	27.75	27	—	—	—	26.25	26.5	27
		L	26	—	26.5	—	26.75	26.75	26.75	26.25	26	—	—	—	25.5	25.25	25.5
	Elevation (degrees)	R	19.5	—	—	—	—	—	28.5	—	21.5	—	19.5	—	20	24	19
		L	5.5	—	—	—	—	—	0	—	0	—	11.5	—	0	4	5
17	Hertel reading (mm)	R	21.5	—	—	—	—	—	21.5	—	22	—	22.25	—	21.75	21.75	21.5
		L	23.75	—	—	—	—	—	26.75	—	26.75	—	27.75	—	27.75	27.75	27
	Elevation (degrees)	R	19	—	—	13	—	—	—	0	—	—	—	—	—	—	—
		L	13	—	—	4	—	—	—	4	—	—	—	—	—	—	—
18	Hertel reading (mm)	R	23.75	—	—	26.5	—	—	—	29	—	—	—	—	—	—	—
		L	28.5	—	—	28.75	—	—	—	29.25	—	—	—	—	—	—	—

prominence of the eye in Graves' disease

afterwards (months)														Remarks
15	16	17	18	19	20	21	22	23	24	25	26	27	28	
—	31	27	29 5	27 5	—	29	—	—	27	—	—	—	—	Remittent phase followed directly on thyroidectomy Gain in body weight concomitant with recession of eyes Remission of ophthalmoplegia relatively more rapid than that of exophthalmos latter perhaps masked by nutritional increment of orbital filling
—	30 5	27	28 5	28 5	—	30	—	—	27	—	—	—	—	
—	22 75	22 75	23 25	22 5	—	23	—	—	22 75	—	—	—	—	
—	23 25	23 75	23 75	23 5	—	23	—	—	22 75	—	—	—	—	
—	—	10 0	10 2	10 0	—	10 1	—	—	10 0	—	—	—	—	
—	—	—	—	—	—	—	—	—	—	—	—	—	—	Thyroidectomy done 2 months after first observation Orbital changes well marked then and progressed afterwards Remittent phase clearly entered but incompletely observed
—	—	—	—	—	—	—	—	—	—	—	—	—	—	
—	—	—	—	—	—	—	—	—	—	—	—	—	—	
—	—	—	—	—	—	—	—	—	—	—	—	—	—	
22	—	—	20	15	15	—	—	—	—	—	—	—	—	Ophthalmic type first attending towards peak of ingravescent stage Sustained remission on left but changes on right becoming static at severe level
28	—	—	30	27 5	27	—	—	—	—	—	—	—	—	
27	—	—	25 5	26 75	27	—	—	—	—	—	—	—	—	
24 75	—	—	24 5	24	24 25	—	—	—	—	—	—	—	—	
—	—	25 5	23	16	22	—	—	—	—	—	—	—	—	Ophthalmic type with conspicuous asymmetry of exophthalmos and ophthalmoplegia No clear indication of remission after 10 months and changes are probably permanent
—	—	5	5	5	3	—	—	—	—	—	—	—	—	
—	—	21 25	20 75	21 25	20 75	—	—	—	—	—	—	—	—	
—	—	27 75	26 75	26 75	26 5	—	—	—	—	—	—	—	—	
—	—	—	—	—	—	—	—	—	—	—	—	—	—	Ophthalmic type Marked asymmetry of exophthalmos at first attendance with subsequent rapid, equal protrusion on right side Ingravescent stage evidently not complete but nearly so on left
—	—	—	—	—	—	—	—	—	—	—	—	—	—	
—	—	—	—	—	—	—	—	—	—	—	—	—	—	
—	—	—	—	—	—	—	—	—	—	—	—	—	—	

this static phase (Fig 1) all had pronounced exophthalmos , 2 exemplified the purely ophthalmic type of Graves' disease, the remaining 4 had been treated for hyperthyroidism in the past

In order to sketch the natural history of the ocular changes we have grouped our material without regard to the presence or absence of hyperthyroidism   Nothing has been observed to indicate that the data cannot be so grouped   But clearly the assumption is made, which seems justified by the evidence (6, 8), that the same kind of eye change is occurring in different forms of Graves' disease *

Phases in ocular changes

Static phase   Fig 1 shows readings of exophthalmos in 6 typical patients observed for periods of approximately 2 years   The values fluctuate irregularly within a range of 2 mm and in each case the data do not represent significant departure from a straight line of zero slope (3)   This narrow range evidently includes errors of measurement as well as natural fluctuations   Thus the latter must be very slight and exophthalmos may be said to be static   This is consistent with its dependence on a definite, measurable increase in bulk of orbital tissues (7)   Marked day-to-day variations in exophthalmos have not been encountered and would be difficult to explain on any known pathological basis   We may presume that a dynamic phase, characterized by the development of exophthalmos preceded the static phase and that remission failed to occur or was incomplete

Dynamic phase   This is illustrated in Fig 2   Case 7 suffered from the ophthalmic form of Graves' disease and attended soon after the onset of eye changes   In Case 8, suffering from thyrotoxicosis, the changes were slight before thyroidectomy but became severe afterwards   In both, the observed cycle approaches completeness   Stages of ingravescence and remission are distinguishable , the former may be defined as lasting until exophthalmos and ophthalmoplegia reach their maximum, the latter, from cessation of ingravescence until both reach their minimum   At the peak of the curves, the rate of change is very gradual and it is convenient to differentiate arbitrarily a stage of maximum protrusion   Remission is usually incomplete, the dynamic phase giving place to the static

An inherent difficulty is that patients first attend at different points in the cycle but recognition of the foregoing stages facilitates interpretation of those in whom data are incomplete   These data, grouped in Table I, can be used to construct curves for individual cases as in Fig 2

* The degree of ocular change is admittedly less in patients with hyperthyroidism (8) But, in any case, the assumption does not render circular a main argument of this paper, that, from the slight effect of thyroidectomy on exophthalmos and ophthalmoplegia in a random sample of thyrotoxicosis, we may conclude that these changes are produced by non thyroid hormone The preliminary account of the natural history is offered as a background against which the behaviour of individual cases after operation may be studied   The ophthalmic group (Cases 7 and 16 18) and the post operative group (Cases 8 10 and 12 15) are closely comparable and their data have simply been compounded to amplify our description of the cycle of eye changes

TABLE II

*Changes in exophthalmos and ophthalmoplegia in Graves disease **

| | EXOPHTHALMOS | | | | OPHTHALMOPLEGIA | | | |
| | Right eye | | Left eye | | Right eye | | Left eye | |
	Mean change (mm)	Range (mm)	Mean change (mm)	Range (mm)	Mean change	Range	Mean change	Range
Stage of ingravescence								
5 patients presenting before, or early in development of ocular changes	+5 2	+1 75 to +8 25	+4 0	+1 75 to +7 25	−29°	−0° to −11°	−20 3°	−10½° to −36½°
9 patients with established changes when first seen	+3 8	+1 75 to +5 5	+2 7	+0 75 to +5 5	−15 3°	−7 5° to −24°	−11 1°	−2° to −24°
Stage of remission								
8 patients†	−2 7	−1 0 to −4 2	−2 0	−1 75 to −3 5	+15 3°	+6 5° to +20°	+10 5°	+5° to +20°

* Values derived from smooth curves drawn as in Fig 2 through serial measurements of exophthalmos and elevation The signs + and − indicate increase and decrease in exophthalmos and elevation, respectively

† In 2 of the 8 (Cases 12 and 15) remission was probably incomplete In 2 other patients (Cases 10 and 11) no remission from maximum protrusion had occurred after 6 months In 2 (Cases 9 and 18) observations were not continued long enough to determine whether remission would occur or not

Extent and Rate of Ingravescence and Remission

Table II shows the extent of changes In patients observed from the beginning an average of 5 mm of proptosis developed with extreme values up to 8 25 mm * It is clear from Table I that exophthalmos may become static at its maximum level (Cases 10, R eye and 11, R eye) though this does not appear to be usual

When recession does occur, its extent is systematically less than that of protrusion Thus, in general, in Graves' disease the eyes do not return to their former position though recession may be sufficient to restore the appearance to normal † In this connection, changes in lid retraction and the width of the palpebral fissure are important

The rates of protrusion and recession are indicated in Table I and Fig 2 In general, they are gradual Progress was relatively rapid in Cases 7 and 8 yet the cycle occupied over 2 years The most rapid rate of protrusion was approximately 1 mm per month (Case 8, right eye) It is usually much slower (average of approximately 0 5 mm monthly), in Case 9, protrusion of 0 25 mm monthly was maintained for 2½ years The ultimate extent of exophthalmos appears not to be significantly correlated to its rate of development Sometimes progress in stair-case fashion is suggested by the distribution of plotted readings (Cases 9 and 10, Table I)

Recession is systematically slower than protrusion , the maximum rate observed was slightly less than 0 5 mm monthly In 3 eyes receding, on an average, by 4 25 mm the rate was 0 3 mm monthly

Symmetry of orbital changes A close symmetry, as illustrated in Case 7, Fig 2, is usual But the onset of clinical signs on one side may be delayed for months or years In Case 8, proptosis on the right was delayed for 4 months but then it developed rapidly In a case shown before the Clinical Section of the Royal Society of Medicine in 1938 as " unilateral exophthalmic ophthalmoplegia," the left eye was proptosed by 5 5 mm and elevation severely limited In 1940, five years after the onset and when the left eye had improved considerably, the right eye protruded, also by 5 5 mm and elevation became completely paralysed Thus one eye may pass the peak of its curve and enter the remittent stage before the other is affected or while the other continues to protrude as in Case 10, Table I Clearly there are several possible ways in which asymmetry of eye signs may develop

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* In a large, random sample of thyrotoxics, ocular prominence was increased beyond normal by 2 1 mm The group probably included many patients without exophthalmos Thus its average extent in thyrotoxics so affected is probably more than 2 mm but less than 5 mm , since the present small group included ophthalmic forms in which ocular changes are generally more severe than in thyrotoxicosis

† Correlative changes in the degree of lid protrusion occur pari passu (9) Such variations in the plane of the globe and lids depend on corresponding increases or decreases in bulk of orbital tissues

Correlation between exophthalmos and ophthalmoplegia

During most of the ingravescent range there is practically a straight line relationship between proptosis and paralysis of elevation * Both may reach their maximum and minimum together, this strict concomitance being observed in two-thirds of cases But ophthalmoplegia may progress more rapidly towards its maximum, reaching its peak and even beginning to remit before the full development of exophthalmos (Cases 7 and 9), conversely remission of exophthalmos may begin before paralysis is maximal (Case 10, left eye) Maximum recovery from ophthalmoplegia usually precedes that from proptosis

In general, the ratio, loss of elevation to protrusion of eye, varies within comparatively narrow limits even though the total extent of the changes differs widely Its value averaged approximately 4 6° per mm (range 3 0°—7 7° per mm) in the cases with steadily progressive changes

In individual cases, however, departures from this correlative behaviour may be observed early and late in the range Thus soon after thyroidectomy ocular prominence may increase by 1-2 mm without decrease of elevation Again, towards its maximum, paralysis of elevation may suddenly become complete It may even be impossible to hold the eye up to the central position This terminal acute paralysis of elevation shows as a distinct dip on the ophthalmoplegia chart (8) , it is not associated with corresponding exacerbation of proptosis Its advent is characterized clinically by marked blinking and watering of the eyes and a pronounced feeling of strain on attempting to look up The patient is only comfortable with the gaze moderately depressed Compensatory torticollis in extension is present

Two other phenomena occasionally associated with extreme overfilling of the orbit, namely prolapse of conjunctiva and marked resistance to pressure back on the eyeball, may be examined briefly

Prolapse of conjunctiva

Conjunctiva prolapsed through the palpebral fissure in 2 out of 10 cases with severe overfilling of the orbit (Cases 10 and 18) The process is illustrated in Case 10, a woman, aged 59, who, before thyroidectomy, showed considerable, bilateral protrusion of the globe and lids Afterwards the right eye continued to protrude while the left receded slightly, Table I

On the right, the lateral fornix conjunctiva became heaped-up and increasingly redundant After 4 months it prolapsed through the palpebral fissure as a clear or greyish, glistening fold, preventing accurate apposition of the lid margins on closure of the eye On the inner side, the lacrimal caruncle and adjacent conjunctiva protruded similarly Between the 4th and 12th months, both folds enlarged and joined below the limbus

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* Scatter diagrams illustrating this and other points mentioned can be constructed from data in Table I and Fig 2.

When the lower lid was drawn down the conjoined folds persisted as a transverse ridge with a sharply-cut ledge where it rested on the lid margin The bulbar conjunctiva below this level appeared compressed between the globe and tightly stretched lid, the conjunctiva of the lower fornix was crowded up by peribulbar fat which extended towards the limbus With continued prolapse the folds became opaque, reddish and firm The superior fornix conjunctiva remained clear throughout and was not redundant

From the 12th month onwards resistance of the eye and conjunctival prolapse steadily decreased, the folds retreating towards their respective canthi By the 22nd month conjunctiva was no longer visible with the eye closed though the caruncle still protruded With the eye open a narrow fold could however be seen towards the lateral canthus On the left side the conjunctiva remained sensibly normal throughout

Thus on the right, conjunctiva prolapsed in association with the ingravescent side of the peak of overfilling and receded as tenseness of the lids and resistance to pressing on the eyeball decreased

Resistance to pressing on the eyeball

An increased resistance to light pressure on the eye has often been noted in Graves' disease patients with severe exophthalmos The development of this resistance was also observed very clearly in Case 10, here the left eye, which fluctuated freely throughout, served as a standard of comparison The right eye became conspicuously firm at the height of ingravescence In Case 8 resistance also increased strikingly and became unyielding when proptosis was maximal In both cases, the palpebral fissure remained narrow and the lids became exceedingly full and tightly stretched Subsequently tenseness of the lids and resistance decreased conspicuously, especially in Case 8 in which considerable ocular recession also occurred

It is clear from other cases in which an equal absolute amount of exophthalmos developed without much increase in tension that the degree of resistance is not precisely correlated to the extent of overfilling Tense lids and palpebral ligaments and a narrow fissure may, by hindering protrusion, combine to increase resistance conspicuously, but in unilateral overfilling without tense lids, resistance is usually distinctly greater on the affected side suggesting that the pathological process also alters the consistency of orbital tissues

EFFECT OF THYROIDECTOMY ON EXOPHTHALMOS AND OPHTHALMOPLEGIA

Observations were made on 39 patients with well-defined thyrotoxicosis treated by thyroidectomy No distinction was made between those with eye signs and those without Thirty-three eyes showed paralysis of elevation before operation, mild in 25, moderate in 5 and severe in 3 * In each patient

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* Standards of ophthalmoplegia are the same as those used previously (8)

TABLE III

Average decrement or increment of prominence and elevation of the eye during the year following thyroidectomy

Interval after thyroidectomy	Actual mean interval for those observed	Number of observations (n =)	Percentage of whole group observed	Mean decrement (—) or increment (+) in exophthalmometer reading	Mean decrement of elevation
1–5 days inclusive	2.0 days	77	100	—0.07 ± 0.1 mm	—
2–11 weeks "	7.5 weeks	57	81	+0.67 ± 0.09 mm	0.9 ± 0.06°
12–23 weeks "	10.0 weeks	65	84.0	+0.91 ± 0.081 mm	0.7 ± 0.06°
24–35 weeks "	29.0 weeks	50	70.8	+1.06 ± 1.72 mm	2.1 ± 0.07°
36–47 weeks "	42.5 weeks	69	89.6	+1.00 ± 1.18 mm	2.1 ± 1.2°
Theoretical complete group in final quarter	37.5 weeks	77	100	+0.00 ± 1.12 mm	—

clinical evidence of hyperthyroidism was completely eradicated by operation , symptoms and signs of mild hypothyroidism supervened in 6

The prominence of the eyes was measured before and at different stages during the year following thyroidectomy   The range of elevation was similarly measured and taken as the criterion of eye movements   The increments or decrements in ocular prominence and elevation obtained in successive quarters were averaged , the values obtained are given in Table III   The right and left eyes are considered together and given equal weight

Ocular prominence   The slight decrement for the immediate post-operative reading is not statistically significant but the subsequent increments all are *   An asymptote to a smooth curve drawn through the increments at successive quarters lies roughly 1 0 mm above the pre-operative base line , thus the average outcome of thyroidectomy is an increase in ocular prominence of 1 mm mostly occurring in the first 6 months after operation

Not all patients were observed during each quarter and it is important to consider how much this may affect the result   In the final quarter 90 per cent were observed   At this stage 5 patients (7 eyes) showed obvious increase in exophthalmos, a development likely to ensure attendance , we may assume that it was not present in the 4 absentees †   Thus there is probably a slight relative excess of patients with ingravescent exophthalmos But this can be allowed for roughly and the excess in mean increment estimated, by including the 4 absentees using the readings obtained when they last attended   The theoretical mean increment thus derived for the whole group is given in Table III , it shows only a slight (and statistically insignificant) difference from the observed increment

The prognosis in individual cases is indicated by the distribution of increments for the two eyes (Table IV)   Except for one patient with severe and rapidly progressive exophthalmos, the increments show a roughly normal distribution about the mean   Moreover, the mean increment for the 34 patients without obvious increase in exophthalmos is not significantly less than that for the whole group   We may conclude that the increase in ocular prominence after thyroidectomy is a consistent finding and that the average result does not depend on the occasional case with obvious ingravescence

Slight protrusion of the lids is associated with the increased prominence of the eye   It is conspicuous when exophthalmos increases markedly but is often apparent in the slighter degrees and puffiness of the lids is not infrequently commented on by the patient herself   Nevertheless she nearly always believes that the eyes have receded   The familiar improvement in appearance after thyroidectomy depends on diminution of lid retraction

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* The differences of the means from zero are greater than 3 times their standard errors

† One of the 4 wrote to say that her eyes were normal , the other 3 were last seen at 32, 30 and 21 weeks after operation when there was no indication of untoward development

TABLE IV

Distribution of the decrements and increments in the final quarter of the year following thyroidectomy

Increment (+) or decrement (—) in mm	—2 0 to —1 25	—1 0 to —0 25	0 to +0 75	+1 0 to +1 75	+2 0 to +2 75	+3 0 to +3 75	+4 0 to +4 75	+5 0 to +5 75	+6 0 to +6 75
Number of eyes *	2	7	26	21	6	3	2	0	2

Mean increment = + 1 0 mm   Range —1 5 to + 6 75 mm

* Total = 69   one eye only measured in a patient with amblyopic divergent squint

TABLE V

Prominence of the eye in controls and thyrotoxics

Clinical group	Number of observations (eyes examined)	Average gain in body weight	Average interval from first measurement	Average change in prominence of eye
Miscellaneous, gaining weight *	22	19 6 ± 16 9 lb (lowest gain = 10 lb)	6 1 months	+0 4 ± 0·42mm.
Simple goitre treated by thyroidectomy	24	1 5 ± 4 9 lb	5·0 months	— 0 1 ± 0 35 mm
Thyrotoxicosis treated by thyroidectomy†	67	14 8 ± 7 7 lb	7 9 months	+1 1 ± 1 35 mm
Thyrotoxicosis treated with thiouracil‡	16	12 0 ± 7 4 lb	3 25 months	+0 73 ± 56 mm

* Neither the gain in weight nor the increment in ocular prominence in this group differs significantly from those in the thyrotoxic series (p=0 1)

† Observations on weight indicating an average gain of 14 8 lb were made at an average interval of 7 9 months after thyroidectomy   therefore the increment for this quarter was taken for comparison

‡ We are indebted to British Drug Houses for supplies of thiouracil

(1, 2, 4, 5, 11) *   The paradox thus obtains that there is generally cosmetic and subjective improvement in spite of slight further filling of the orbit with consequent protrusion of the globe and lids †

* It is generally stated that some improvement in exophthalmos also follows thyroidectomy but the only systematic effort previously made to measure ocular prominence before and afterwards appears to be that recorded by Soley (11)   He concludes that prominence increases in over 50 per cent of patients and decreases in only a small percentage

† In a sample of thyrotoxics, in whom thiouracil adequately controlled hyperthyroidism, the same ocular effect was obtained namely improvement in lid retraction but slight further protrusion of the globe and lids (Table V)

Range of elevation This shows a mean decrease of 2 4° in the final quaiter (Table III) The decrement is small compared with the average range of 36 2° in thyrotoxicosis (8) Six patients showed major trends in the values (trend group), 5 worsening and 1 improving Simultaneously, identical trends occurred in their readings for exophthalmos

Omitting this trend group the mean decrement in the final quarter is reduced to 1 5± 0 4° This represents a true statistical difference between the measuiements before and after thyroidectomy but whether it has pathological significance, is doubtful *

The behaviour of patients in the trend group may be interpreted in relation to the natural cycle of eye changes It is clear from data in Table I that the whole or any part of the cycle may follow thyroidectomy

Discussion

Non-thyroid oiigin of exophthalmos and ophthalmoplegia In a random sample of thyrotoxicosis we show that there is little average change in prominence and elevation of the eye after thyroidectomy In individuals in whom exophthalmos and ophthalmoplegia were pronounced before operation there is further progress or remission afterwards, according to the point of the cycle reached The observations suggest that these phenomena are not produced or even potentiated by excess thyroid hormone In the ophthalmic form, the causal agent is clearly some non-thyroid hormone and presumably the same substance is responsible for exophthalmos and ophthalmoplegia in fully developed Graves' disease Further the variable behaviour of individual cases after operation suggests that its duration and intensity of action are not much affected by eliminating hyperthyroidism

Bulk changes in orbital tissues in Graves' disease It has been shown (7, 9) that the position of the globe and lids depends on the bulk of orbital tissues The present curves of exophthalmos are consistent with its resulting from a true growth change in those tissues The development of the post-thyroidectomy increment is also gradual and probably depends on a similar mechanism The general rate of increase of exophthalmos, as indicated by the gradient of curves, is of the same order in all cases and in individuals remains uniform over long periods and when over-filling becomes severe Thus there is no suggestion that the causal process differs in mild and severe degrees The change is evidently reversible though the deciescent phase is slower and less complete, the bulk of orbital contents may indeed become static at its maximum level

* P is less than 0 01 No systematic difference in method of measurement was observed to develop during the experimental period But, in general, lid retraction is substantially less at the end of one year from thyroidectomy and consequently less of the globe's surface is exposed for making readings with the vertometer The change would not affect measurement of small ranges of movement but may bias slightly the values for full rotation especially upwards

The post-thyroidectomy increment of ocular prominence, 1 0 mm , corresponds to a mean increase of 0 7 c c in bulk of orbital tissues (7)   The increment is not characteristic of thyroidectomy, qua thyroidectomy, for it does not occur after operations for simple goitre (Table V) , nor do these patients gain weight after operation   Recovery from wasting may be the relevant factor   It is known that emaciation from sundry causes is associated with decrease of orbital tissue and recession of the eye (7)   Recovery of weight is accompanied by ocular protrusion (Table V), and thyroxine therapy in the experimental animal causes wasting of orbital tissues (10)

It has been shown that in Graves' ophthalmopathy, the correlation between decrease of elevation and proptosis is usually close and averages about 4 5° per mm   The low post-operative ratio for decrement of elevation to increment of prominence, 1 5° to 1 mm in the non-trend group, would suggest that after thyroidectomy a different factor is at work   It seems probable that part at least of the post-operative increment is due simply to recovery from wasting

It is clear that exophthalmos and increase in bulk of orbital tissues in thyrotoxicosis are generally associated with loss of body weight , this is exemplified in Case 11, Table I   The reverse phenomenon, recession of the eye and decrease in orbital tissue associated with gain in body weight, is shown in Case 8, Fig 2 and Case 14, Table I   Also, in the ophthalmic form, the bulk of orbital tissue may increase by some 20 per cent while the body weight remains practically constant (Case 7, Fig 2)   We may conclude that in Graves' disease, non-thyroid hormone exerts a disproportionate influence on the bulk of the orbital tissues and that this can far outweigh the local nutritional effect of hyperthyroidism or its elimination

Summary of Conclusions

1   Dynamic and static phases occur in the natural history of exophthalmos and related eye signs in Graves' disease   The former, characterized by stages of ingravescence and remission, precedes the latter

2   The average extent of exophthalmos in different forms of Graves' disease is approximately 2-5 mm   Its rate of development averages about 0 5 mm monthly   Paralysis of elevation usually develops concomitantly, 4°-5° being lost for each 1 mm protrusion   Remission of both exophthalmos and ophthalmoplegia is systematically slower and less complete than ingravescence   Maximum recovery from ophthalmoplegia precedes that from proptosis

3   The curves of development and remission of exophthalmos are consistent with their dependence on growth changes in orbital tissues   These appear to be produced by some non-thyroid hormone which exerts a disproportionate effect on orbital tissues

4 In patients with thyrotoxicosis the prominence and range of elevation of the eye show little average change after thyroidectomy There is further slight protrusion of the eye probably due to recovery from wasting which suggests that thyroid intoxication may to a slight extent combat the enlargement of orbital tissues caused by non-thyroid hormone

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THROMBOSIS OF THE FEMORAL ARTERY WITH MYOHÆMOGLOBINURIA AND LOW SERUM POTASSIUM CONCENTRATION

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RENAL damage following burial beneath debris in air raids is due to ischæmic muscle necrosis, and is associated with the passage of myohæmoglobin in the urine (11)   It has occurred with soft compression for periods of two hours (9) as well as with the more violent crush associated with fallen masonry (10)   Cases have been recognised where a similar condition follows rupture, spasm or obstruction of a main limb artery, as the result of traffic and other civil accidents (5, 20, 21, 36, 48 and unpublished data)   In such cases muscle necrosis is seen   only in two of these cases, however, has myohæmoglobin been identified in the urine   Other cases showed acid hæmatin, which might be derived from either hæmoglobin or myohæmoglobin   It seemed possible that a similar lesion might be found following non-traumatic occlusion of arteries supplying muscle   Such a case is recorded here in the hope that it will stimulate interest in the more general aspects of these primarily local problems

Case history

A woman aged 63, with uneventful previous history, had noticed some recent diarrhœa and loss of weight , three weeks before entry she had had a slight cough and cold   Two days before admission she felt a cramplike pain in the right leg on getting out of bed , the pain has been continuous since The leg became cold, blue and numb, but she was able to walk on it for the remainder of that day, after which she took to her bed   *Examination* showed a wasted woman, confused and rambling in her statements, and incontinent of urine and fæces, temperature 100°F, pulse 104/min , respiration 20/min   There was loss of skin elasticity   the tongue was dry and fissured

Optic fundi normal, no enlarged glands were felt   Heart apex beat 5 cm from midline in 5th space , apical systolic murmur   B P 120/70 mm Hg The liver was not palpable , both kidneys could be felt   The right leg was pale, cold and slightly cyanotic up to the upper margin of the patella, the tendon reflexes were absent, and no arterial pulsation could be felt beyond Hunter's canal   The left leg showed normal reflexes and good pulsation (by oscillometer and by palpation of arteries)

Next day, (fourth day from onset of leg pain) B P 120/70 mm Hg , the zone of coolness and pallor had extended 2 5 cm higher , the oscillometer showed no pulsation below the middle of the thigh but the femoral pulse was felt in the groin   Urine on this and succeeding days was slightly pink and gave a positive benzidine reaction, although it contained no red cells No spectral bands could be seen until the protein was concentrated by freezing   The concentrated urine showed clearly the bands of myohæmo-globin at 581 m u , shifting to 578 m u on the addition of carbon monoxide The urine was acid (pH 5 6), contained albumin (90 mg per 100 c c) and excessive creatine (Fig 1)   Over a period of 22 hours, 1 3 mg potassium and 23 mg chloride (as NaCl) were excreted per hour   Blood taken on this day showed   blood urea, 123 mg per 100 c c , serum protein 6 1 g per 100 c c , plasma inorganic phosphorus 5 mg per 100 c c , and a low potassium, 6 7 mg per 100 c c (2 estimations in duplicate)   By the fifth day after onset, the line of demarcation had spread up to 5 cm below Poupart's ligament   the limb was packed in ice   Plasma inorganic phosphorus 4 9 mg per 100 c c , serum protein 6 5 g per 100 c c   After consultation with Professor Grey-Turner and Mr Franklin, it was decided to delay amputation until her general condition improved   After two days' ice treatment, she was much more rational and less confused   Her appetite improved and blood urea fell   Urine, after the eighth day, gave no benzidine reaction   An electrocardiogram on the eighth day showed multiple ectopic foci with paroxysms of auricular tachycardia   the normal beats showed right axis shift with depression of the S-T segment in leads 2 and 3, and almost flat T waves in lead 1   On the tenth day the blood urea had fallen further and the serum potassium risen slightly , plasma sodium was decreased to 288 mg per 100 c c , plasma protein 5 7 g per 100 c c , plasma chloride (as NaCl) 614 mg per 100 c c , plasma CO_2 combining power 53 volumes per 100 c c   Her pulse was only occasionally irregular   Chest X-ray showed a large shadow in the left posterior mediastinum   On the twelfth day a bedsore had developed , the right heel was showing signs of mummification   Between the eighth and twelfth days inclusive, 2 33 mg potassium were being excreted per hour , chloride (as NaCl) on ninth, tenth and twelfth days at 16, 30 and 22 mg per hour respectively   Thirteenth day , plasma protein 5 5 g per 100 c c , plasma chloride (as NaCl) 610 mg per 100 c c , plasma CO_2 combining power 55 volumes per 100 c c , serum sodium 316 mg per 100 c c   the blood urea had fallen and the potassium risen farther (Fig 1)   The line of demarcation had not shifted   On the

fourteenth day Professor Grey-Turner performed a disarticulation through the hip joint under spinal anæsthesia All exposed veins and arteries except the obturator were thrombosed The muscles attached to the iliac crest contracted on stimulation only in their uppermost cm below this was a pallid zone, and again below this the muscle was friable and necrotic and showed many petechial hæmorrhages Contraction was seen on cutting
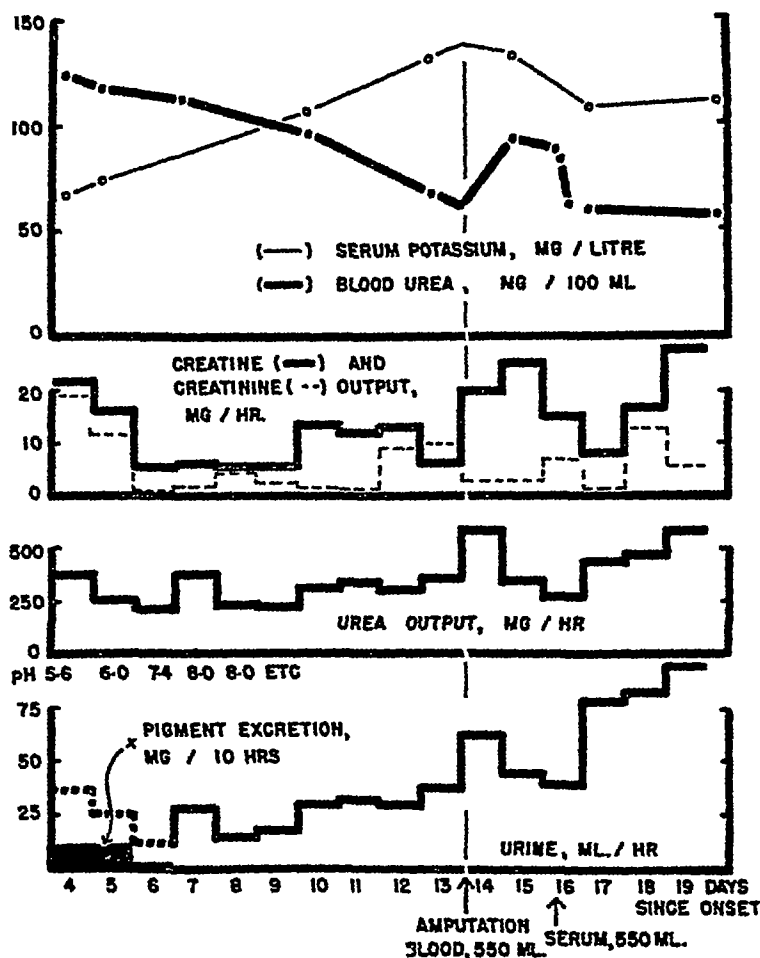
Fig 1 Chart showing clinical course

the adductor group lower down (supplied by the obturator artery) Slight oozing of dark blood occurred from the iliac bone when sawn across

On incising the femoral artery of the amputated leg a recent antemortem thrombus was seen extending distally for 15 cm , and adherent to the walls of the vessel Below this, for a further 18 cm was an older, slightly paler

clot, densely adherent, its lower end projecting into the lumen of one of the small arteries supplying the knee joint Below this, the lumen of the artery was free from blood, except for two clots lying free, the heads consisting of pale platelets, the tails of darker and more stringy clot issuing from a tributary The veins were thrombosed only in the upper portion of the limb The muscles were necrotic in most of their extent, showing many dark petechial hæmorrhages on a pale ground, and were unduly friable There were some areas of normal muscle at the top of the amputated limb (about 4 cm), between this and the necrotic part was an area of pallid muscle without hæmorrhage about 2 5 cm wide

Microscopically, the vessels showed adherent thrombosis with normal vessel walls Muscle showed necrosis with reactive changes in the pallid boundary zone similar to that seen in crushing injury Biochemical analysis of the muscle is given in Table I

Though negligible blood loss occurred, 560 c c of blood was given during operation and 800 c c of 5% glucose saline in the following 3 hours Next day, the pulse was strong, but blood pressure measured only 85/60 mm Hg Blood urea was slightly increased again

On the 16th day, the pulse was strong, occasional dropped beat, B P 90/60 mm Hg No visible venous distension or pulsation in neck at 45° 550 c c of recalcified citrated plasma (Clegg and Dible (14)) was given with no increase in blood pressure despite a rise in venous pressure to 5 cm in the neck veins at 45°, falling to the preceding level in two hours Hæmoglobin, 11 2 g per 100 c c before, fell to 9 8 g per 100 c c immediately after transfusion, rising to 10 1 g per 100 c c seven hours after Next day it was 9 3 g per 100 c c and two days later 10 4 g per 100 c c Urea clearance 9 6 c c per min = 24 per cent of normal Potassium excretion, 5 mg per hour from fifteenth to eighteenth days inclusive , chloride excretion (as NaCl) 78 mg per hour on the eighteenth and nineteenth days Over the next four days, diarrhœa reappeared, with incontinence , the posterior flap became gangrenous, and although the B P was maintained at 90/60 mm Hg , she became drowsy and died, twenty days after the onset of pain in the leg Blood urea was still slightly raised on the nineteenth day, 56 mg per 100 c c , and serum potassium low, 11 mg per 100 c c plasma chloride (as NaCl) 500 mg per 100 c c and blood sugar 92 mg per 100 c c The urine had been alkaline since the sixth day (pH 7 4 — 9 0) and contained between 20 and 50 mg protein per 100 c c

Autopsy three hours after death showed an extensive area of superficial sepsis at the operation site and large bedsores over the sacrum The lower lobe of the left lung was shrunken and adherent, due to occlusion of its bronchus by a papilliform fleshy tumour Distal to this, the bronchial branches were distended by pus and the lung parenchyma appeared gangrenous The liver was enlarged (2730 g) by numerous massive metastases measuring up to 10 cm in diameter The kidneys were not enlarged (320 g together), then capsules stripped easily, leaving a smooth

surface   The cortex showed an indistinct pattern of half its normal
thickness   the medulla appeared normal   The spleen (170 g) pulp was
diffluent   The right common iliac artery and vein were filled with ante-
mortem blood-clot   The left femoral artery was empty and the upper
10 cm of the left femoral vein was occluded by antemortem clot   No other
significant abnormality was found

    Microscopical examination confirmed the diagnosis of bronchial
carcinoma

    The kidneys showed well-developed arterio-sclerotic ischæmic changes
The arcuate vessels showed thickening and multiplication of their elastic
laminæ and the cortex showed radial areas of atrophy corresponding to the
interlobular arterial distribution   The interlobular vessels showed similar
changes   The distribution of these changes was uneven, some areas being
much more affected than others and the superficial parts of the cortex more
altered than the deep   Although a few of the changes in certain nephrons
might be the late results of crushing injury renal lesion, the widespread
arteriolar ischæmia made it impossible to satisfy oneself that this was really
their cause

    The adrenal glands showed no abnormality apart from a diminution
in cortical lipoid

Discussion

    General effects of muscle ischæmia   Since technical developments have
allowed surgeons to remove clots successfully from arteries, many cases of
arterial embolism have been published   While attention has been focussed
mainly on the local condition, it is evident that there may be general
manifestations

    Thus, for instance, death occurs in the majority of cases treated without
operation, in 116 out of 123 cases of aortic embolism (19, 29, 43 and 44),
and in 24 out of 27 cases of peripheral limb vessel embolism (32)   Cases
treated by embolectomy are more frequently published, especially if they
recover , nevertheless, Nystrom in 1936 (39) showed that while recovery
occurred in 86 cases after embolectomy and in 69 cases after amputation,
227 cases died after operation   Since then, others (1, 4, 7, 22, 28, 30, 31,
32, 33, 34 and 38) have published 24 cases which died after embolectomy
and 19 after amputation , 43 cases recovered after embolectomy and 13
recovered after amputation

    Pearse (40) reviewing 282 cases of embolectomy showed that while
function recovered in 40 per cent of those whose emboli were removed in
the first ten hours, only 14 per cent of those operated on between ten and
twenty hours, and only 8 per cent of those operated on between twenty
and thirty hours, recovered the use of their limb   75 per cent of those who
did not recover the good use of their limb died   Thus patients seen late,
in whom embolectomy will probably be unsuccessful and who now die, form

a very large group   While some of these deaths were due to further emboli affecting organs such as brain, intestines or kidneys, many seem related only to limb ischaemia and resultant gangrene   No observations relevant to the mechanism of death in such cases have been recorded   Albuminuria, haematuria and uraemia have been occasionally observed, and were then usually attributed to renal infarction   While this has often proved to be correct at postmortem, another possible cause exists   It has been shown that when a limb is crushed for some hours, for example by fallen masonry, the muscle becomes necrotic and the limb itself shows anaesthesia, paralysis and skin erythema or blisters ,   Volkmann's contracture may develop on recovery   All these have been recorded after arterial embolism (22)   A comparison of these two types of limb ischaemia might reveal the mechanism whereby necrosis of large amounts of muscle produces death, and thus facilitate effective treatment in the two conditions

Hitherto only one case of arterial embolism has been studied in the light of information gained in crushing injury   We are indebted to Dr Guthkelch for further details of a case he has already briefly described (23)

A female aged 54, with auricular fibrillation for many years, developed embolism of the right femoral artery   Twelve hours later a clot was cleared from the profunda artery, but the superficial femoral artery was full of clot and in spasm   The adventitia was stripped, but gangrene developed with a demarcation line just below the knee joint   The urine output was very small after the operation, averaging 250 c c per day for the first five days She then became incontinent   The blood urea was 144 mg per 100 c c on the second day, and despite potassium citrate, 1 3 g hourly for four doses, then two-hourly started on the second day, it rose to 268 on the fourth day and to a maximum of 328 mg per 100 c c on the fourteenth day   On the seventeenth day an isotonic sodium sulphate intravenous drip was set up and over the next four days 9 1 were given   This brought the blood urea to 40 mg per 100 c c by the twenty-third day   On the thirtieth day, amputation was performed in the thigh and she died suddenly twenty-four hours later, no autopsy being obtained

In experimental crushing injury the general effects due both to plasma leaving the bloodstream and to substances entering the bloodstream from the damaged area, were found to be proportional to the amount of tissue damaged (12)   The same seems to be true within rather wider limits for crushing injury in man (9)   Grant and Reeve have also formed the opinion (personal communication) that, in cases of industrial, traffic and air-raid trauma, the degree of general disturbance was related not only to the amount of blood lost but to the volume of tissue damaged   In the case under consideration here the volume of tissue damaged was large (one leg = 20 per cent. of body weight), but the general effects were surprisingly slight compared with a lesion of similar size occurring as a result of " crushing injury "   Thus, although the patient was seen two days after the onset, and while the thrombosis was still progressing up the limb, there was no

limb swelling, and no sign of plasma loss   the amount of pigment put out
in the urine while under observation was very small, and there was only
moderate rise in blood urea, such as could be explained by increased
katabolism with a pre-existing mild ischæmic kidney lesion   Whether any
further renal damage occurred after the thrombosis is not known   The
reason for the slightness of this general reaction is that the uptake of
substances from the damaged area depends on the extent and rate of re-
establishment of the circulation   In experimental limb ischæmia, due to
tight binding, and in most air-raid casualties, the circulation is re-established
to the ischæmic area within an hour of release, leading to swelling of the
limb and the appearance of muscle products in the urine

In the present case re-establishment of the circulation was confined to
the narrow boundary zone between living and dead muscle   Moreover,
thrombosis advanced up the limb, thus abolishing the circulation through
areas in which it was being re-established   The muscle analysis in Table I

TABLE I

Muscle analysis

Muscle	Water g/100g wet wt	Potassium mg/100g wet wt	NaCl mg/100g wet wt	Pigment mg/100g wet wt	Position of band * m.u.
Left sartorius postmortem (normal)	79 2	270	138	—	—
Right quadriceps above knee (amputation totally necrotic)	79 1	140	347	1390	578 1
Pale portion of right quadriceps (boundary zone)	77 2	73	467	667	578 1 –

* Hb = 578 0
MyoHb = 581 0

shows that only in the boundary zone had the intracellular electrolytes
been replaced to any great extent by chloride   tissue obtained from the more
peripheral parts of the limb, although completely dead, had lost only about
half its potassium content   The analysis shows that the blanched area
contained an amount of pigment which is normal for muscle , this pigment,
however, appeared to be hæmoglobin spectroscopically, and is accounted
for by the thrombosis and diapedesis seen microscopically   In the more
peripheral muscle sample, the amount of pigment is more than double the
usual amount, measured by the alkaline hæmatin method , spectroscopically
this too showed the bands of hæmoglobin

Serum potassium concentration   It may be calculated from the above figures that something like 2 to 3 g of potassium was lost from the affected muscle during the 20 days   yet from the fourth day onwards only very small quantities were put out in the urine, about 1 g in the 16 days thereafter During this time the patient was ingesting most of the ordinary ward diet, probably about 1 g potassium daily as a minimum figure   During this time, therefore, she must have been in positive potassium balance, although fœcal output was not measured   This is said to be about 300 mg daily (41) Except towards the end she had no diarrhœa in hospital   Yet the serum potassium level was found to be, to start with, about one-third of the normal level   Levels as low as this are due either to excessive storage or excessive loss of potassium

Excessive storage of potassium occurs clinically in familial periodic paralysis (2), where it is associated with weakness and decreased K output in urine   No relation to muscle ischæmia has been found except in a case of Buerger's disease in a male aged 25 with creatinuria and absent arterial pulse in the right leg (46)   It is also seen after the administration of testosterone propionate (8) and after glucose and insulin (27), in the latter case accompanied by decreased urinary output, a fall in serum phosphate and an increase of phosphate in the muscles   The patient's very low urine potassium output, despite adequate intake, is in favour of storage   It is not thought, however, that the cause was one of the three just mentioned, but that storage followed and was secondary to a primary excessive loss

Excessive potassium is lost after desoxycorticosterone (18)   this results in deficient renal tubular resorption of potassium, the resultant fall in serum level being prevented by nephrectomy   Excessive potassium loss may be due to primary loss of base, as in sprue with diarrhœa and low serum calcium and phosphorus (25), and in chronic renal damage (6) even when there is little nitrogen retention, the base deficit being ascribed then to failure of the ammonia mechanism in the damaged tubules   Excessive loss of potassium is found also associated with other conditions such as gastrointestinal obstruction and vomiting (3,17), where loss of water and electrolyte leads to increased plasma bicarbonate   it is associated with increased plasma bicarbonate in Cushing's syndrome (35, 49, 50) and other conditions (unpublished data)   In conditions with excessive loss in the urine, the tissue K concentration has been found low (18), and a test dose of potassium is almost entirely retained (49)   Urinary loss of potassium, but not usually to such a degree that will produce low serum levels, occurs in dehydration alone without primary base loss (16)   This loss is in excess of that due to protein katabolism, and is accompanied by loss of cell water, and an " outlying " acidosis (45)

The patient under discussion had renal impairment and had had diarrhœa   There was no chloride or phosphate deficiency and no alkalosis, but the serum potassium and sodium levels were low   The most probable explanation is that excessive amounts of potassium had been lost, together

with cellular water from dehydration (diarrhœa and diuresis from renal damage) and base loss from preceding acidosis with failure of the ammonia mechanism   during rehydration there was retention of potassium by the cells at the expense of the body fluid level, and retention of both chloride and potassium by the body, leading to low urine output of both substances   Support to this theory is given by the muscle analysis   the potassium concentration in undamaged muscle was lower than usual, 2 7 g /kg wet weight against 3 3 g /kg (average of thirteen human pectoral samples ranging from 3 1 to 3 5 g /kg (37))   Part of this reduction is accounted for by œdema, since the chloride space (extracellular water) is 256 c c /kg instead of the more usual 154 c c /kg (15)   However, potassium still tends to be low calculated as concentration in intracellular water, 128 m eq /l * compared with a normal of 138 m eq /l (calculated from data of Mangun and Myers (37) on human traffic accident deaths, assuming 150 c c /kg extracellular water), or 143 m eq /l in the monkey (24)

The multiple ectopic foci and the low voltage T waves shown in the electrocardiogram can be ascribed to the low potassium level (13)   An unexplained observation was the low blood pressure uninfluenced by increasing venous pressure and venous return   No observations on the possible effect of neoplastic tissue on potassium metabolism have been found on record

SUMMARY

(1)   Myohæmoglobin was found in the urine following thrombosis of the femoral artery in a woman with carcinoma of the bronchus   The muscle in the affected limb showed macroscopically, microscopically and biochemically a picture very similar to that seen following crushing injury

(2)   The blood urea, high initially, remained above normal throughout, but the kidney was the seat of severe ischæmic change

(3)   Potassium concentration was greatly reduced in the serum and in the urine, and slightly in the cells of undamaged muscle   It is suggested that the low serum potassium was due to base depletion and dehydration consequent on preceding diarrhœa and renal damage

APPENDIX

The following biochemical methods were used —

Urea (blood and urine) blood sugar serum protein, potassium (serum, urine and muscle), serum sodium plasma inorganic phosphorus, King, Haslewood, Delory and Beall, Lancet, 1942, 1, 207

Plasma chloride   King Haslewood and Delory, Lancet 1937, 1, 886

Plasma CO$_2$ combining power   Van Slyke and Cullen, J Biol Chem., 1917, 30 347

Urine protein   King and Haslewood, Lancet, 1936 ii, 1153

Urine myohæmoglobin   Bing and Baker, J Biol Chem., 1931 92, 589 , Bing, J Biol Chem., 1932 95 387

Urine creatinine and creatine , Folin, J Biol Chem , 1914, 17 469

Muscle pigment   alkaline hæmatin method of Clegg and King Brit Med J., 1942, 2, 329

Muscle and urine chloride   Van Slyke, Sendroy et al J Biol Chem., 1923, 58, 523

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* This has been calculated by the method of Hastings and Eichelberger (25) with a Gibbs Donnan ratio of 0 96 and a 1 per cent solid in ultra filtrate   No correction was made for fat, collagen or blood

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THE NATURE OF THE CIRCULATORY CHANGES IN
BURN SHOCK *

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IT has been shown (3, 8, 9) that there is considerable local fluid loss following burns, and it is thought that this local fluid loss is largely responsible for the state of shock following burns   This explanation is not always adequate (6) for it has been shown recently that burn shock can occur with insignificant local fluid loss (19)   The degree of local fluid loss in burned rats was determined by comparing the weights of the burned and the control side   It was found that a 10 second burn at 65°C of one hind limb caused considerable local œdema but shock did not occur   Burning a limb at 100°C to 150°C for 15 seconds to two minutes produced no visible œdema, yet the animals died.   The amount of measurable local fluid loss was considerably less in animals burned by this method than in the slightly burned animals which did not develop shock   Furthermore, it was observed that in animals with the more severe burns, those that had recovered had more œdema than those that died   It was, therefore, apparent that the state of shock following the more severe burn was not due to local fluid loss, but must have been due to some other factor, presumably either a nervous or humoral change   It is the purpose of this paper to identify this factor in the production of burn shock not due to local fluid loss

Experiment I

The role of the nervous system

If nervous impulses from the burned area to the brain, or from the brain to the burned area contribute to the shock state, then it should follow that animals with severed spinal cords should have no or less shock than control animals   The experiment was performed as follows   A transverse incision about 2 cm long was made on the back of etherized rats at the level of the lowest rib   The animals were dorsi-flexed and the cords crushed by small bone crushing forceps   Little bleeding occurs if the large ventral

blood vessels are avoided. Immediately after recovery from the anæsthesia, there is complete paralysis and anæsthesia below the level of the incision, the animals seem perfectly normal from the waist up and crawl around by using their front extremities Control operations were performed on etherized rats by crushing both front extremities with the same forceps It was thought that this produced a degree of tissue trauma and hæmorrhage approximately equal to that produced by severing the cords After recovery from the preliminary operation, the lower extremities of both groups of animals were burned at 100°C for 15 sec (21), a type of burn previously found to cause shock with insignificant local fluid loss It was found (Fig 1) that there was no difference in survival time or mortality in the denervated as compared with the control series

EFFECT OF SECTIONING THE SPINAL CORD ON BURN SHOCK,
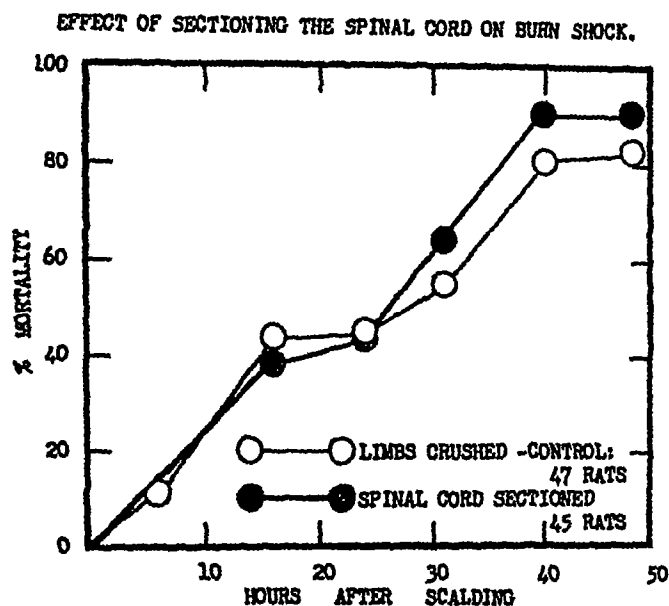
Fig 1 Effect of severing the spinal chord on burn shock Twenty four hours after operation, rats were scalded over lower one half of body at 100°C for 15 sec Denervation has no effect on either mortality or survival time

This experiment demonstrates that the nervous system is not directly implicated in the pathogenesis of this type of burn shock, but it does not rule out a humoral factor affecting the central nervous system with secondary systemic changes Experiments designed to test this possibility will be presented later (Experiment IX)

Since we have already ruled out the role of local fluid loss, it follows that the shock state must be due to a humoral change resulting from the burn This type of shock will be termed " toxic " This term is not meant to imply that a new substance has been formed, it is possible that it may be a quantitative chemical change which produces the toxic effect For purposes of convenience, the term " extravasative burn shock " will be used to describe the shock following considerable local fluid loss

Experiment II

Bleeding volume in burn shock

It was observed that the appearance of animals with extravasative and toxic burn shock was similar, both groups having cold skin, increasing asthenia, and dying in a similar manner   Since it is now generally believed that the effective circulating blood volume is reduced in shock, we thought it would be of interest to compare the bleeding volumes of the two groups of shocked animals

Thirty-five normal animals were anaesthetised with ether, a longitudinal incision was made on the abdomen and a 20 gauge needle inserted into the abdominal aorta   A syringe containing a small amount of dry heparin was attached to the needle and the blood withdrawn until the animal was
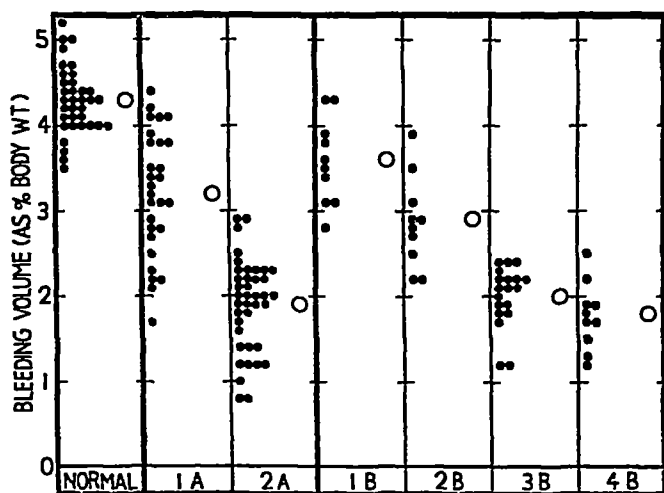
Fig 2   Bleeding volume following burns which lead either to toxic (A) or extravasative (B) burn shock   Group 1A   both hind legs scaled at 100°C   for 45 sec., bled 24 hr later   Group 2A   both hind legs plus groin scalded at 100°C   for 2 min   bled 3 to 5 hr later   Group 1B   scalded to head at 60°C for 10 sec., bled 24 hr later   Group 2B   scalded to head at 65°C for 10 sec   bled 24 hr later   Group 3B   scalded to head at 70°C for 10 sec   bled 3 to 5 hr later   Group 4B   scalded to head at 75°C for 10 sec., bled 3 to 5 hr later   The dots represent single rats and the large circles the group average   This figure demon strates that the bleeding volume is reduced in both types of burn shock, and that the reduction is proportionate to the degree of trauma

dead   The volume of spilled blood was measured by sponging the peritoneal cavity with cotton and the amount of this blood was determined by weighing the cotton before and after the absorption of blood. In computing the bleeding volume, the value for the spilled blood was added to that obtained in the syringe, which was also determined by weight   The bleeding usually lasted from 2 to 4 minutes   It was found that the bleeding volume of normal

etherized rats was fairly constant, being $4\,3 \pm 0\,4\%$ of the body weight *
Similar values have been obtained in dogs (10)

Fig 2 illustrates the changes in bleeding volume occurring in different
degrees of toxic and extrasavative burn shock   Two degrees of burns of
both limbs producing toxic shock were used, 100°C for 45 sec (1A), and
100°C for 2 to 3 min (2A)   The bleeding volume of Group 1A was
determined 24 hours after the burn and Group 2A, 3 to 5 hours after the
burn   Thirty-nine animals with burns of greater duration (2A) had bleeding
volumes averaging $1\,9 \pm 0\,7\%$ of the body weight   Twenty-eight rats
with less severe burns (1A) had bleeding volumes of $3\,2 \pm 0\,7\%$ The
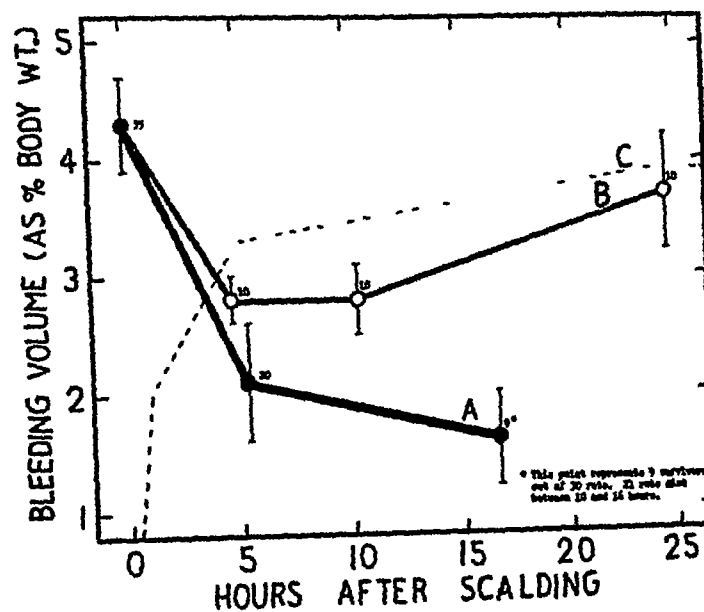
Fig 3   Relation of bleeding volume to time after scalding following trauma of various degrees
of severity   Curve A   scalded up to head at 70°C for 10 sec   Curve B   scalded lower one
third of body at 70°C for 10 sec   Curve C   rate of local fluid loss due to scalding one side
at 75°C for 10 sec , the ordinate for this Curve should read " Percent Fluid Loss " , the
details of the construction of this curve have been presented elsewhere (19)   The numbers
accompanying each point represent the total rats in the mean, the vertical lines represent
the standard deviation of a single observation   This figure demonstrates that following a
severe burn there is a progressive diminution in bleeding volume, whereas following a less
extensive burn, after an initial decrease, there is a gradual increase in bleeding volume as
the animal recovers

bleeding volumes in the extravasative type of burn are shown in Groups 1,
2, 3 and 4B (Fig 2) in which the animals were scalded up to the head for
10 sec   In Group 1B, the average bleeding volume of 10 rats scalded at
60°C was $3\,6 \pm 0\,5\%$ of the body weight , in Group 2B, 10 rats scalded
at 65°C , $2\,9 \pm 0\,4\%$, in Group 3B, 19 rats scalded at 70°C , $2\,0 \pm 0\,4\%$,

* In the experiments reported in this paper, bleeding volume has been calculated on the
basis of % body weight   It is realised that the use of body surface instead of body weight would
be more satisfactory   However, the values obtained do not fluctuate greatly because rats were
chosen with a weight range rarely over 10% in any experiment

and in Group 4B, 10 rats scalded at 75°C, 1 8 $\pm$ 0 4%   This experiment clearly demonstrates that there was a reduction in the bleeding volume in both types of burn shock, and that the reduction in both types is proportionate to the degree of trauma   Harkins has also observed a reduction in bleeding volume in burn shock (10)

Experiments were performed to determine whether there is any correlation between the bleeding volume and degree of shock   Following severe burns, as the degree of shock becomes greater, this reduction of bleeding volume becomes progressively more pronounced (Fig 3)   Following a slight burn, after the initial reduction, there was a progressive increase in bleeding volume during recovery (Fig 3)

It would seem that the deleterious state of the shocked animal can be accounted for, in part at least, by the loss of effective circulating blood, since the loss is considerable   Whatever the cause of this loss may be, fluid replacement therapy should prove beneficial in both types of burn shock

Experiment III

Differences in hæmo-concentration

The reduction of effective circulating blood in extravasative burn shock can be explained by the local loss of plasma, thus accounting for the high hæmoglobin and hæmatocrit determinations so often found in burned subjects   In the following experiment we have found this explanation holds only in the extravasative type of burn shock   The hæmoglobin (Sahli scale) determinations of 29 normal animals averaged 63 (40 to 80)%   Hæmoglobin determinations of 19 animals, 2 hours after a burn at 65°C for 10 sec up to the head, averaged 73 (45 to 97)%   In 28 animals the hæmoglobin was determined before and after scalding up to the head at 75°C for 10 sec , and it was found that the average increase was 20 (0 to 50)% (Fig 4, 1B)   The hæmo-concentration in this type of burn is only temporary , in 24 hours the hæmoglobins of similarly burned animals have returned to normal levels (Fig 4, 2B)   This would seem to be due, in part, to the withdrawal of fluid from the tissues into the blood stream, restoring the decreased blood volume   It is unlikely that this late dilution of the blood comes from re-absorption of the œdema, for in 24 hours no reabsorption has taken place (Fig 3, Curve C)   This dilution was not exogenous since no food or water was allowed after the burns

The hind legs of twenty animals were burned at 100°C for 2 min , a type of burn producing toxic shock   In striking contrast to the hæmo-concentration found in extravasative shock, there was only a slight increase in hæmoglobin at the end of 3 to 4 hours (Fig 4, 1A), and in 24 hours there was actually a decrease (Fig 4, 2A)   Anæmia has been previously described 24 hours following burns (22)   This reduction in hæmoglobin in the last 10 to 15 hours of the 24 hour period would seem to be due, in part, to the

same factor described above, 1 e , transfer of fluid from the tissues into the blood stream

The statement, so often made, that the degree of shock can be measured by the degree of hæmo-concentration is not always correct, for as we have shown, in toxic burns, hæmo-concentration may never occur and the animal usually dies with a reduced hæmoglobin value   There is also no correlation between the bleeding volume and the degree of hæmo-concentration   These experiments offer an explanation for the differences of opinion regarding the presence of hæmo-concentration and hæmo-dilution after burns   Both occur, but at different times and under different circumstances   The
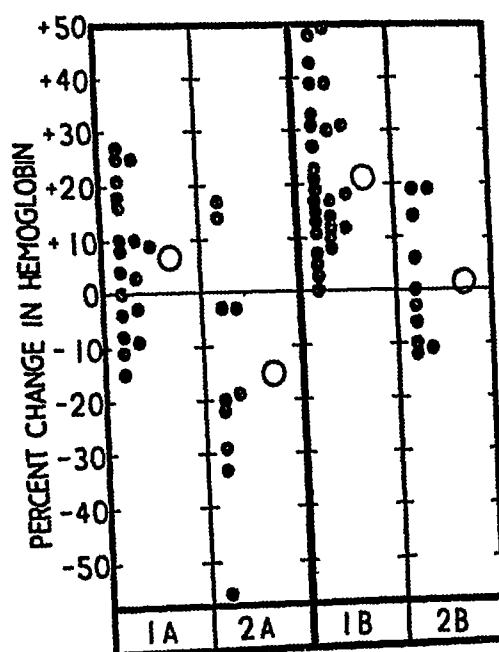
Fig 4   Percentage change in blood hæmoglobin in toxic (A) and extravasative (B) burn shock   Group 1A   both hind legs scalded at 100°C for 2 min , final blood 3 to 4 hr later , Group 2A   both hind legs scalded at 100°C for 45 sec , final blood 24 hr later , Group 1B   scalded to head at 75°C for 10 sec , final blood 3 to 5 hr later , Group 2B   scalded to head at 60°C for 10 sec , final blood 24 hr later   The dots represent single rats and the large circles the group average   This demonstrates that in early toxic shock there is slight or no hæmo-concentration ,   whereas, in early extravasative shock, considerable hæmo concentration takes place   In 24 hours there is dilution of the blood in both groups so that in extravasative shock, the hæmoglobin values fall to normal and in toxic shock, anæmia may be present

experimental burns here employed produced sharply contrasting pictures of extravasative and toxic shock   Clinically it is likely that both types occur in varying degrees in the same burned individuals   Furthermore, the factor of local fluid loss would appear to be more important in human burns in which there is usually weeping from burned skin   These considerations account for the frequent finding of hæmo-concentration in severe human burns, although cases have been reported which had severe burns with little or no hæmo-concentration (1)

Experiment IV

Increased blood content of viscera in burned animals

The important problem of where the blood goes in toxic shock immediately arises   It is clear that in toxic burn shock both red cells and plasma are lost from the effective circulation, since there is little or no hæmo-concentration   We have previously shown that there is little local fluid loss in this type of burn (19)

It has long been known that the viscera may have a congested appearance in shock, although some have denied that this occurs (23) In this series of experiments, we were impressed with the congested appearance of the viscera   But it was felt that quantitative studies must be performed before final conclusions could be drawn   In attempting to solve this problem, various organs were weighed and the amount of blood
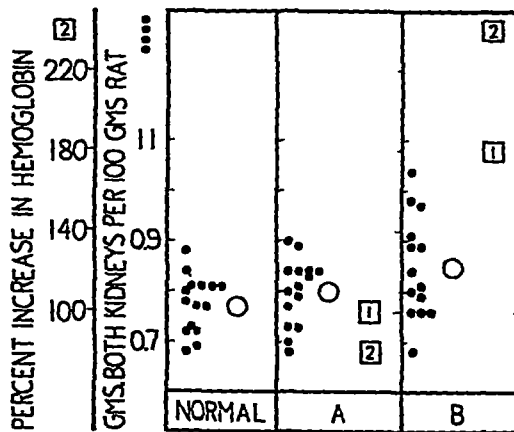
Fig 5   Weight and hæmoglobin contents of kidneys in burn shock after exsanguination Group A   scalded to head at 70°C for 10 sec   Group B   both hind legs scalded at 100 C for 2 min   The dots represent the weights of both kidneys per 100 g of body weight and the large circles the average kidney weight of the group   The squares represent percentage increase in hæmoglobin content of 6 to 10 kidneys compared with an equal number of normal kidneys as a standard   The figures within the squares are the experiment number   *See* Fig 6

in each was estimated after exsanguination as described in Experiment II Fifteen normal rats, 15 rats scalded up to the head at 70°C for 10 sec (extravasative shock), and 14 rats scalded up over both hind extremities at 100°C for 2 min (toxic shock) were exsanguinated 5 hours after the burn The kidneys and livers were then removed and weighed. Each organ was then cut transversely by a standardised method and 6 to 10 kidney sections

from each group were put in 50 c c of distilled water and 4 to 5 liver sections from each group were put in 100 c c of distilled water The next day the haemo-globin contents of the bloody supernatant fluids from the 3 series were compared colorimetrically * Following the extraction with water, the tissues of both normal and burned animals were very pale, indicating that haemoglobin had been mainly removed It was found (Fig 5 and 6) that the organ weights of the two series of burned animals were not abnormal, but that the number of red blood cells which exuded and were extracted from the cut surfaces of the organs of the animals in extravasative and toxic shock was considerably higher than that of the control organs (Fig
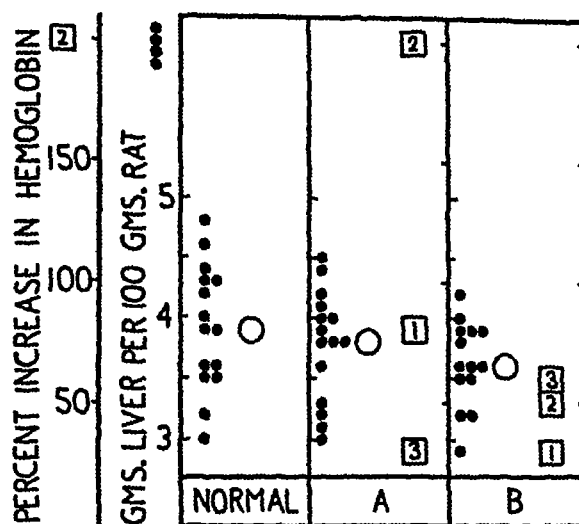
Fig 6 Weight and hæmoglobin content of liver in burn shock after exsanguination Groups A and B same as in Fig 5   The dots represent the weight of single livers per 100 g of body weight and the large circles the average liver weight of the group The squares represent percentage increase in hæmoglobin content of livers of shocked animals compared with an equal number of normal livers as a standard The figures within the squares are the experiment number In this figure and Fig 5 a marked increase occurs in volume of blood in the kidneys and liver of shocked animals Yet, due to dehydration, there is little change in organ weights

5 and 6, A and B) The increased blood content of the organs of the burned animals must have been due to vasodilatation of certain vessels But since the weight of the organs was not increased, the extra blood retained must have been balanced by dehydration It is to be remembered that these animals were exsanguinated after 5 hours, allowing time for dehydration of the tissues

* The details of, and errors inherent in, this method will be presented elsewhere The estimation is only semi-quantitative

Experiment V

The importance of exsanguination

If a rat is killed with ether and sufficient time to allow coagulation of the blood is permitted before the organs are removed, it is found that the organs of shocked animals appear similar to, or slightly more congested than, the organs of non-shocked rats

The difference between the gross appearance of the organs of shocked and non-shocked animals becomes manifest if the animals are killed by exsanguination, when certain organs of the non-shocked animals become very pale, whereas the same organs of the shocked animals remain unchanged or pale only slightly   The kidney shows this difference well (Fig 7)   The importance of exsanguination before examining the tissues in shock does not appear to have been previously considered, and this fact may explain the failure fully to appreciate the vascular factor in shock   For, if the animals are not exsanguinated, the appearance of the organs may not be particularly distinctive

It is evident that even following this stimulation of hæmorrhage, the blood vessels in certain organs of shocked animals do not constrict, but contain large quantities of blood with resulting loss from the effective circulation

Experiment VI

The capillary lesion

The following experiment was performed to find out which vessels retain the blood in the viscera of shocked animals, and the degree of this retention

Extravasative and toxic shock were produced as previously described After 5 hours, the animals were exsanguinated from the abdominal aorta , normal animals were exsanguinated in the same manner   Thick slices of various organs were dropped into 10% formalin   After 2 days, sections were made through the centre of each slice and the tissues stained with hæmatoxylin and eosin *   This procedure allows minimal loss of red blood cells in the fixative   The sections were examined by Dr R Strauss who did not know whether any particular slide was from a normal or a burned animal In 18 out of 20 tissues from normal animals, the examiner stated that the tissues contained minimal amounts of blood   In 24 out of 27 burn cases, he described congestion, and an increase in the number, of capillaries It was thought that the venules were also dilated, but it was difficult to be sure because of the frequency with which the cells were washed out of the

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* 2 to 3 drops of strong ammonia water were added to the second 95% alcohol dehydrating medium   2 to 3 drops of glacial acetic acid were added to the eosin solution. This causes more vivid staining of the red blood cells

TABLE I

Capillary counts and widths in kidneys of burned as compared with normal rats

Procedure	Glomerular area		Tubular area	
	No of open capillaries per field *	Width (microns)	No of open capillaries per field *	Width (microns)
Normal, after exsanguination	6, 7, 7, 6 6, 7, 10, 10 13, 13, 9, 12	7 to 14 7 to 14 7 to 14	25, 15, 24, 20 25, 20, 20, 20 10, 27, 15, 16	7 to 14 7 to 14 7 to 14
90% of body surface scalded at 100°C for 20 sec, bled 5 min. after burn	30, 35, 32, 35 45, 40, 45, 40 30, 35, 34, 35 39, 50, 60	7 to 42 7 to 42 7 to 42 7 to 42	65, 65, 65, 65 50, 40, 70 40, 40 60, 50, 60, 100	7 to 42 7 to 63 7 to 42 7 to 42

* Magnification 430×

vessels in the preparation of the sections The arteries and arterioles appeared empty in both the shocked and normal animals Capillary counts were done with the results shown in Table I It can be seen that there are 2 to 3 times as many open capillaries in the shocked organs and the diameters of the capillaries are greatly increased This change was especially prominent in the adrenal, kidney, liver, intestine, and heart

The red blood cells in many capillaries appear quite firmly packed This can best be explained by the fact that plasma is propelled more easily than cells (5) Thus, a greater proportion of red blood cells than of plasma is taken out of the effective circulation This is undoubtedly an important factor in the dilution of the blood found in Experiment III It would appear that the administration of whole blood might be more beneficial than the use of plasma for the treatment of this type of shock in its later phases when hæmo-dilution is present

Experiment VII

The vascular factor in the venous return

The reduction in bleeding volume demonstrated in Experiment II would appear to be an excellent measure of the degree of circulatory failure For, if one cannot get any more blood out of the abdominal aorta while the heart is still beating, it is obvious that at this point the tissues do not receive any blood and there is no longer any venous return to the heart Now, two factors may be concerned in the flow of blood from the periphery to the heart, namely, the contraction of the heart and the tone of the blood vessels It seemed important to determine which of these factors is concerned in the venous return in order to have a better understanding of the manner in

which the bleeding volume is reduced in shock If the heart is concerned in its own venous return and the blood vessels play only a passive role, then it should follow that the bleeding volumes of animals without hearts would be greatly reduced If, on the other hand, the blood vessels themselves propel the blood to the chest, then the removal of the heart should have little or no effect on the bleeding volume In order to determine which of these factors is concerned with the venous return, the following experiment was performed in normal rats under ether anæsthesia

The thorax was opened by cutting through the sternum with large scissors The heart was lifted up by its apex and all the large vessels were severed The volume of blood collected in the thorax from the open vessels was measured by weight after absorption on cotton

The bleeding volumes of 10 normal rats exsanguinated by removing the heart averaged 4 1 $\pm$ 0 3% of the body weight as compared to 4 3 $\pm$ 0 4% for 35 normal rats bled from the abdominal aorta Of 24 rats in toxic burn shock, the average bleeding volume following removal of the heart was 2 5% of the body weight

These results demonstrate that the venous return during exsanguination is not due to the gradient produced by the contractile power of the heart It would appear to be mainly due to the contractile power of the blood vessels themselves for the blood continues to flow into the chest from the severed vena cavæ for some time after the heart is removed and the amount of blood which can be recovered by this method is essentially the same as that recovered from the aortæ of animals with intact hearts In shock, on the other hand, this contractile power of the blood vessels is diminished so that during exsanguination the blood remains in the capillaries causing the reduction in the effective circulation

This experiment, like others to be reported elsewhere (18), does not support Henderson's theory of the failure of the veno-capillary system in shock, since he postulates that the failure of the venous return is primarily due to loss of muscle tone (13) We have shown that retention of the blood occurs in organs not surrounded by striated muscle such as the kidney and liver, and in these organs at least, the retention of the blood must be due to a disturbance in the capillaries themselves

Experiment VIII

Capillary atony, the primary disturbance

The suggestion has been made that although there is congestion of the viscera in shock, this may be terminal and due to anoxia (17) In the preceding experiments, congestion of the viscera was found early in shock, after 5 hours in experiments where many of the animals could have been expected to live four times as long In order to rule out completely anoxia as the cause of visceral congestion, the following experiment was performed.

If congestion occurs in the first few minutes following trauma, it is obviously not due to anoxia   Three groups of 8 rats were severely burned up to the head for 20 sec at 100°C   This is an extremely severe burn , the animals rapidly go into shock followed by death within 12 min (average 10 rats)   No visible œdema occurs   1, 2 and 5 min after the burn the animals were exsanguinated by removing the heart, and the bleeding volumes were determined   The red blood cell content of the kidneys was determined colorimetrically and sections were made of the organs   The viscera appeared grossly congested in all three groups of animals   This was especially noticeable in the lungs, kidneys, liver, and intestines   There was a con-
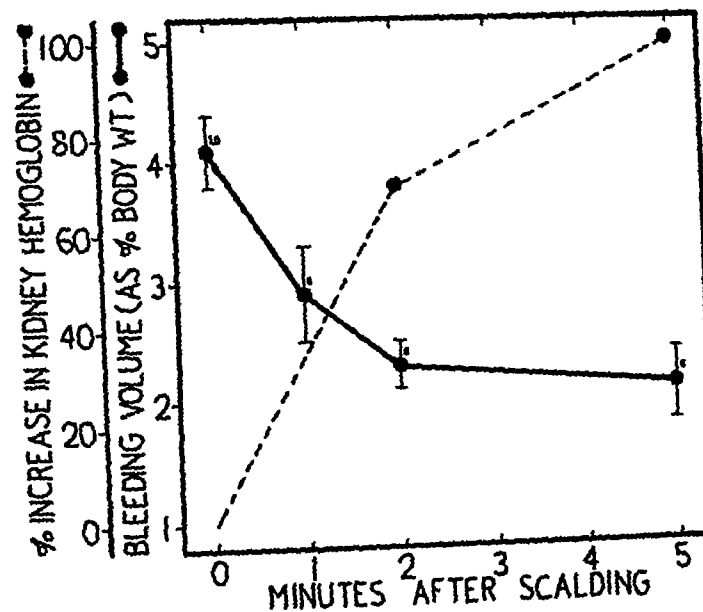
Fig 8   Effect of an extremely severe burn on bleeding volume and percentage increase in kidney hæmoglobin in relation to time after scalding   Rats were scalded to head at 100°C for 20 sec   The numbers accompanying each point represent the total rats in the mean , the vertical lines, the standard deviation of a single observation   This demonstrates that in one minute after the burn, the bleeding volume is already significantly decreased   In two minutes, the hæmoglobin content of the kidneys has increased almost 75%

spicuous reduction of bleeding volumes and retention of the red blood cells in the viscera in all three groups   These changes are graphically shown in Fig 8   In 1 min after the burn, the bleeding volume is about 29% less than that of normal animals, the red blood cell content of the kidneys is increased, accounting for the diminution of bleeding volume (Fig 8)   In 2 min (8 animals) the bleeding volume is further reduced and the viscera more congested

If an animal is severely burned in this manner and the abdomen immediately opened the onset of the visceral congestion can easily be

observed   This is especially noticeable in the kidney which can be seen becoming larger and engorged   Proof that these visceral changes are actually occurring is offered in Experiment IX

Experiment VIII demonstrates clearly that the visceral congestion in shock is not terminal nor due to anoxia, for it happens quickly after the burn   The capillary atony, therefore, must be the primary disturbance

It has been suggested that the visceral congestion observed following severe burns may be caused by over-heating the organs themselves as a direct result of burning   It was found necessary to control this point since we observed the intraperitoneal temperatures to rise as high as 110°F following this severe type of burn   Following the less severe types of burns described in this paper, hyperpyrexia in the viscera did not occur

The following observations show that visceral hyperpyrexia is not the cause of the congestion which occurs after severe burns   Forty c c of saline at 111°F were injected into the peritoneal cavity of several normal rats   The saline was allowed to remain for four minutes, after which the animals were exsanguinated.   It was observed that the kidneys were normally pale as they are in non-shocked animals   The same experiment was performed except that the saline was injected at 134°F   At the end of four minutes the intraperitoneal temperature was 111 2°F   The animals were then exsanguinated and it was observed that the kidneys were pale Thus, visceral temperatures much hotter than those following a burn of 100°C for 30 sec do not cause the capillary atony observed in the experiment

In order to obtain more information on this subject, it was felt important to produce shock from a very severe burn without visceral hyperpyrexia By injecting a cool, inert liquid intraperitoneally, a relatively low temperature in this region can be maintained during and after a severe burn   If under these circumstances the viscera still are congested, it would offer further evidence that the visceral congestion is a direct result of the shock state and not of hyperpyrexia

This experiment was done in the following manner   Forty c c of mineral oil at 71 6°F were injected intraperitoneally into a 200 gram rat   The animal was burned at 100°C for 30 sec up to the head   At the end of four minutes, the intraperitoneal temperature was 100 8°F   The animal was exsanguinated and it was found that the kidneys were dark and congested and contained more than a normal amount of hæmoglobin by extraction This experiment was repeated 4 times with the same results   The visceral congestion was not due to the direct effect of the mineral oil because it was found that the intraperitoneal injection of mineral oil without burning did not produce any visceral congestion

Experiment IX

The humoral agent acts peripherally

In Experiment I, it was shown that animals with crushed spinal cords develop the same degree of toxic shock following burns as do non-denervated animals This experiment proved that the shock state is not due to nervous impulses going to the brain from the burned area, or to the burned area from the brain via the spinal cord It was concluded that this shock state was " humoral " in origin The possibility remained, however, that the circulating humoral agent could act through the central nervous system In order to determine whether this possibility was responsible for the shock state, the following experiment was performed

Under ether anæsthesia, through a lumbar incision, the right kidney was removed and the wound sutured The animal was then severely burned up to the head at 100°C for 30 sec This produced immediate profound shock Two to three min after the burn, the left kidney was removed It was found that the kidney removed before the animal was burned became pale and small and the cortex became wrinkled because the blood vessels contracted and discharged blood from the organ The kidney which was connected with the systemic circulation for 2 to 3 min following the burn was considerably larger and more congested than its control and remained enlarged and congested after discharging varying amounts of blood Thus, it is clear that the vessels contained in a normal kidney, after it is removed from the body and thus from its connections with the central nervous system, contract and empty themselves of blood, whereas, this ability is largely lost in the shocked kidney which remains engorged under the same circumstances Thus, the humoral agent prevents to a large extent, the contraction of the blood vessels in the shocked kidney Since this defect is present when the kidney has been removed from the body and is thus unconnected with the central nervous system, it follows that the humoral agent must act locally, and independently of the central nervous system Fig 9 demonstrates the differences in appearance in normal and shocked kidneys removed from the same animal

In this experiment, each animal supplied both the control and the shocked kidney By knowing the difference in weight between the two organs, the amount of blood which was trapped in the shocked kidney was determined This experiment was performed 10 times and it was found that a shocked kidney can trap an average of $0 11 \pm 0 09$ c c of blood This is a large amount of blood and if all the tissues retained an equivalent amount, it can be estimated that at least 100% of the circulating blood volume would be lost from effective circulation Thus the retention in the kidneys must be greater than in other tissues These measurements of the large amounts of blood which can be trapped in the organs demonstrate in a clear manner that the capillary atony is a direct cause of the reduction in the bleeding volume

ANGINA PECTORIS AND TOBACCO

By G. W. PICKERING and P. H. SANDERSON

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THE importance of so-called tobacco angina lies not in its frequency, for it is rare, but in the belief, to which its description has given rise, that tobacco may constrict the coronary arteries thus adversely affecting the heart In this paper we present three cases in which smoking would favour or precipitate attacks of angina pectoris, and observations on one of them to elucidate the part played by tobacco

A summary of past writing reveals that true angina of effort occurring in heavy smokers and stopped by abstaining from tobacco has been described rarely Allbutt (2) mentioned a medical man who smoked too many cigarettes and experienced a constricting pain across the middle of the chest on walking up a hill, if he stopped the pain passed off The tobacco was discarded and with it the pain departed Fiessinger's (8) third patient had retrosternal pain on walking, which disappeared when he gave up smoking but returned when he took it up again White and Sharber (27) encountered 3 such cases, one with bundle branch block, and 2 with no other evidence of heart disease Other cases of angina of effort have been described in which the attacks could be produced by smoking Gallavardin (9) mentions 2 such cases, in the first, one pipe after his evening meal would certainly bring on an attack Ralli and Oppenheimer (23) refer briefly to six patients " beyond middle age and predisposed to angina pectoris in whom the attacks could be provoked very promptly by smoking a cigarette", the pain caused by smoking was relieved by nitroglycerin and not by a placebo Such cases are probably similar to these here reported, but the descriptions are disturbingly brief In addition 3 cases have been described by Fiessinger (8) and 1 by Clendening (5) in which smoking produced an attack described as anginal and in which the patient died after a period varying from a few minutes to 48 hours No evidence is available as to whether death was due to cardiac arhythmia, or to coronary thrombosis, if indeed it was due to either

But many of the cases reported as tobacco angina have little resemblance to angina of effort Two of Gallavardin's (10) cases, Lian's (18) two cases,

and Moschcowitz's (21) four, all conform to a type in which attacks of severe precordial pain, often radiating to the left arm, occur at rest in heavy smokers and cease to occur at a varying period, often months, after tobacco has been given up   One of Gallavardin's cases may be quoted as an example

Between the ages of 23 and 24 the patient had 7 or 8 attacks of violent gripping left sided thoracic pain radiating down the left arm to the little finger , the attacks of pain occurred at rest and were preceded by vertigo or abdominal discomfort and feebleness of the legs   He had been a heavy smoker since the age of 17, but when the attacks began he gave up smoking altogether for a few months and then began again, smoking only two cigars daily   The attacks remained frequent for a fortnight, then gradually diminished, and finally ceased about a year after the first attack   During the year in which he experienced the attacks he noticed no pain or breathlessness on walking   Between the ages of 25 and 40 he had several brief attacks of paroxysmal tachycardia   When finally seen at the age of 47, the heart was normal to physical and radiological examination

The later occurrence in this patient of brief attacks of paroxysmal tachycardia raises the question as to whether the anginal attacks in this and similar patients could have been due to a disturbed rhythm of the heart   For as is evident from Bristowe's (4) description, and as Barnes and Willius (3) and others have emphasised, anginal pain may occur in paroxysmal tachycardia and, as Mackenzie (19) showed, in other forms of abnormal rapid heart action   Some cases present features which support this view   Thus both Gallavardin's (10) patients had cerebral disturbances, even loss of consciousness in the first   One of Huchard's (13) patients, a soldier aged 48 and a heavy smoker had attacks of a painful oppression in the chest radiating to both arms and the left little finger , these attacks occurred on walking too quickly and at night and were often preceded and announced by violent palpitations , the attacks ceased 15 months after stopping smoking and the administration of potassium iodide   As Barnes and Willius (3) pointed out it is often very difficult to identify attacks of paroxysmal tachycardia unless the attacks are witnessed , their case 6 was admitted to hospital four times for attacks of precordial pain and it was only when, on the last occasion, she was seen in an attack that the nature of the condition became evident.   Of all the recorded cases of tobacco angina we have found only one in which an attack was witnessed , this was Allbutt's (2) first case in which the patient, a strong man of middle life, writhed in distress in an attack lasting some three or four minutes   " To note the behaviour of the heart during the height of the seizure was impossible , but as it declined the action was rapid and irregular   Moreover in the tranquil intervals more or less arhythmia continued, an arhythmia having the common characters of tobacco heart "   That tobacco may cause a disturbance of cardiac rhythm was clearly Allbutt's view and is that of most writers to-day   Yet there is little evidence published in support ,   Cowan and Ritchie (7) mention a lawyer aged 45 in whom smoking a single cigarette would induce extrasystoles

Nor do animal experiments help much to provide a clear cut answer   Clerc and Pezzi (6), investigating the action of nicotine on the hearts of anæthetized dogs, encountered a variety of disturbances of rhythm, including auricular fibrillation and nodal rhythm , but they specifically stated that extrasystoles were not common, and that paroxysmal tachycardia of a ventricular origin occurred only in the later stages of the drug's action On the evidence available it is clearly impossible to assign a mechanism to this group of cases of so-called tobacco angina, in many of which indeed it is uncertain on the one hand whether the pain was of cardiac origin and on the other whether the occurrence of the attacks in smokers was more than a chance coincidence   It is our purpose merely to indicate that they are clinically distinct from the condition dealt with in this paper and to point out that their nature remains obscure

Case Reports

Case 1   J J , a clerk, aged 53 years, of German Jewish origin, first experienced, about six months previously, a pressing cramp-like pain just to the left of the lower half of the sternum, on walking to his office in the morning   The pain got so bad that he had to stop and rest, the pain disappearing in about a minute, after which he was able to complete his journey   He subsequently had pain under the same circumstances each morning, and also noticed it when walking for similar distances, or upstairs The pain was not associated with nausea, choking, or other sensations , it never radiated into the arms or neck , it never occurred when he was completely at rest   On several occasions he has noticed that a few puffs of a cigarette have brought on an attack of pain identical with that experienced on walking , this has rarely been with the first but usually with about the third cigarette of the day   These attacks have nearly all occurred in his office where his work is of a responsible nature   He admits that on many of these occasions he has been more or less agitated by some problem connected with his work, and on some of these he has been pacing up and down his office   Only on one occasion has pain come on in his office when he was not smoking   This was five weeks ago, when after considerable excitement there was an attack of pain which lasted ¾ hour and in which he felt he might lose consciousness , after the attack he felt nothing abnormal He has smoked between 5 and 10 cigarettes daily for years   One month ago, being convinced that smoking was bringing on the pain he gave it up   Since then the amount of exercise which induced the pain has not changed

He has been healthy until the present illness   His father had hypertension and arterio-sclerosis, and died aged 73   His mother died of hemiplegia, aged 78

The patient was a small man, a little fat, with quick rather abrupt movements   He was intelligent, co-operative and accurate in his statements He presented no clinical or radiological evidence of enlargement of the heart and no signs of cardiac failure   The heart sounds were normal as was the

fundus oculi   The lungs were normal to physical and radiological examination   The urine contained no protein or sugar.   The blood hæmoglobin was 90% (Haldane scale) and the Wasserman reaction negative   Extrasystoles were frequent at first, disappearing after exercise   The electrocardiogram showed left axis deviation in limb leads, with a biphasic T wave in lead I, lead CF 4 showed an inverted T wave and a biphasic QRS complex   An experimental investigation of this case is described later

Case 2   A H B , a commercial traveller, aged 63 years, led an athletic life, playing championship lawn tennis till 5 years ago, and playing 3 long sets 3 years ago   He was a very large man, 5ft 11in tall, weighing 14½ stone   18 months ago he noticed a dull ache about the middle of the chest when on route marches with the Home Guard   At first he bore it, but later had to give up marching   Since then he has developed pain after progressively less exercise until just before admission he was unable to walk more than 100 yards   When the pain comes he has to stand still until it passes off, which it does in about 2 minutes   This pain is felt in an area about the size of a hand in the mid-sternal region and is accompanied by a peculiar soreness in the left wrist which has caused him to change his wrist watch to the right side , it is not accompanied by breathlessness or palpitations   He is much more susceptible to pain just after a meal   Thus a few months ago, he could walk 2 miles without pain if he went out at noon , but at 2 o'clock, shortly after lunch, pain would come on after walking 500 yards   Two or three times recently he has had the usual pain very slightly when at rest after a large fatty meal   External temperature does not affect the exercise he can take   He smoked 2 oz of pipe tobacco a week until 4 years ago when he changed to cigarettes of which he smokes 15 a day, inhaling the smoke   Smoking has never brought on the pain at rest and he never smokes while walking, but the pain is much more liable to come with exercise if he has smoked just before   Thus he frequently takes his wife to the cinema in the afternoon   If he has a light lunch and does not smoke after it, he can walk to the cinema without pain , if he does not smoke during the performance he can walk back without pain , if, however, he smokes 3 or 4 cigarettes in the cinema he always has pain on the walk home

The day before admission while emptying a bucket, he had a very severe attack of pain slightly more to the left than usual and involving the whole forearm   The pain was very intense, continued even when he got into bed and lasted over 24 hours , he vomited about one hour after it began   It was followed by the usual sequelæ of cardiac infarction, namely pyrexia to 101 4° lasting 7 days, a leucytosis of 19,000 with 81% of polymorphonuclear cells, and a raised sedimentation rate   The hæmoglobin was 80% on the Haldane scale   Examined three weeks after the infarct, the heart was enlarged radiologically and clinically, the impulse being in the 4th space 5in from the midline, but there was no valvular disease   The arterial pressure which had been 145 systolic and 100 diastolic on admission had fallen to 130/95.   The urine showed no abnormalities

Case 3   The third patient, a medical man now aged 64, has cardiac symptoms which, undoubtedly, in his view, are aggravated by tobacco He was a fairly heavy cigarette smoker in a busy operating and teaching position   In an attempt to limit smoking he made it a rule not to smoke until the evening about 5 to 6 p m   He was smoking fairly continually from then until midnight   He had his first definite anginal attack in 1931 at the age of 51   This came on after a heavy operating list at hospital, followed by a lecture, then sudden exertion following an irritating circumstance   He got a sharp attack of sub-sternal pain that radiated into his left arm and ring finger   It was specially severe in his left forearm. Since that date any untoward exertion, especially walking fast on a hill, would provoke a reminder, though not so severe as on the first occasion In the summer of 1939 when on holiday and smoking more than usual the pain was frequent and annoying and one day it was especially persistent After this date, August 1939, he stopped tobacco completely , the incidence of pain gradually diminished and after a few months he could take rather more exercise with impunity and in fact for three years was practically free   He has had some more pain lately in spite of not smoking but this may be due to overwork owing to war conditions and to increasing age   His pressure is normal, there are no heart changes and his urine is free from albumin X-ray of his chest shows a slight broadening of his aortic arch and left sided hypertrophy, but not excessive

On three occasions since he gave up tobacco he has attempted to smoke and each time the pain has returned   On the first occasion he was dining with some colleagues and had some wine   All his friends were smoking and he joined them   He had smoked about half of the cigarette when he got quite a definite attack   He had not had any pain for some weeks, was not thinking of the possibility of it and is quite certain it was not psychological The other two occasions were somewhat similar and in each case the pain manifested itself before the cigarette was finished   The pain is always the same, sub-sternal radiating into the left arm and ring and little fingers and is identical to the pain he gets after over exertion

Observations on the production of anginal pain

In the following experimental analysis of the effect of smoking on the production of anginal pain in Case 1, the procedure introduced by Wayne and Laplace (26) has been followed closely   The observations were made in a warm quiet room on 6 separate mornings, between 10 a m and 12 30 p m , the patient having been seated for 20 min before the experimental period began

Exercise   The relationship to exercise was typical of angina of effort On 14 occasions, after at least 20 min rest, the patient walked up and down a 2 step staircase similar to that described by Wayne and Laplace (26) until the pain began, when he at once sat down, and readings of blood pressure
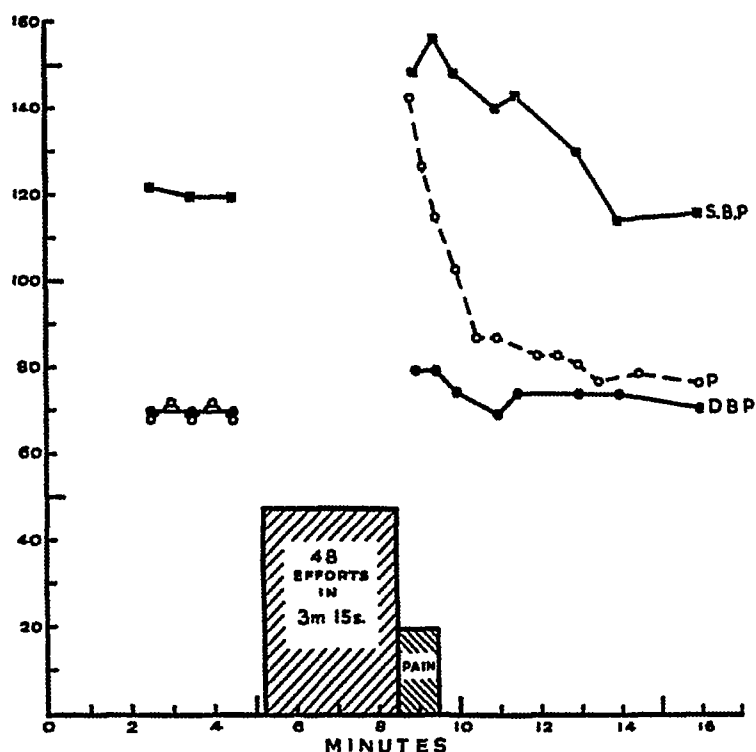
Fig 1   Shows the effect of exercise in producing pain and the accompanying changes in blood pressure and pulse rate   In this and subsequent figures the squares and solid line represent systolic blood pressure, the solid circles and solid line diastolic blood pressure, in mm Hg, and the open circles and interrupted line pulse rate in beats per min
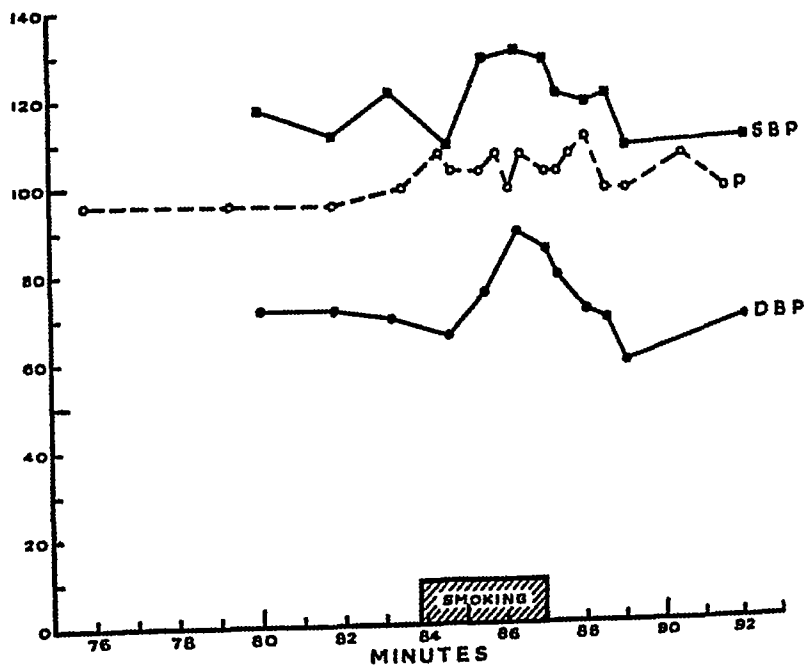

Fig 2   Shows the effect of smoking 1 cigarotte (Player's " medium ") on the blood pressure and pulse rate

and pulse rate were resumed   The number of efforts* required to produce pain was remarkably constant, varying from 42, at a rate of 1 effort in 3 9 sec , to 52, at a rate of 1 effort in 4 1 sec   After sitting down, pain gradually lessened, disappearing after between 50 and 70 sec in 12 of the 14 experiments, and 40 and 90 sec in the remaining 2   The first reading of pulse rate was between 136 and 164 in 10 of the 14 experiments, rather slower or faster in the remainder   The first reading of arterial pressure always showed a rise of the systolic value, but the diastolic pressure was rarely above the resting level   These changes in pulse and blood pressure gradually returned to normal (Fig 1, 3 and 4)
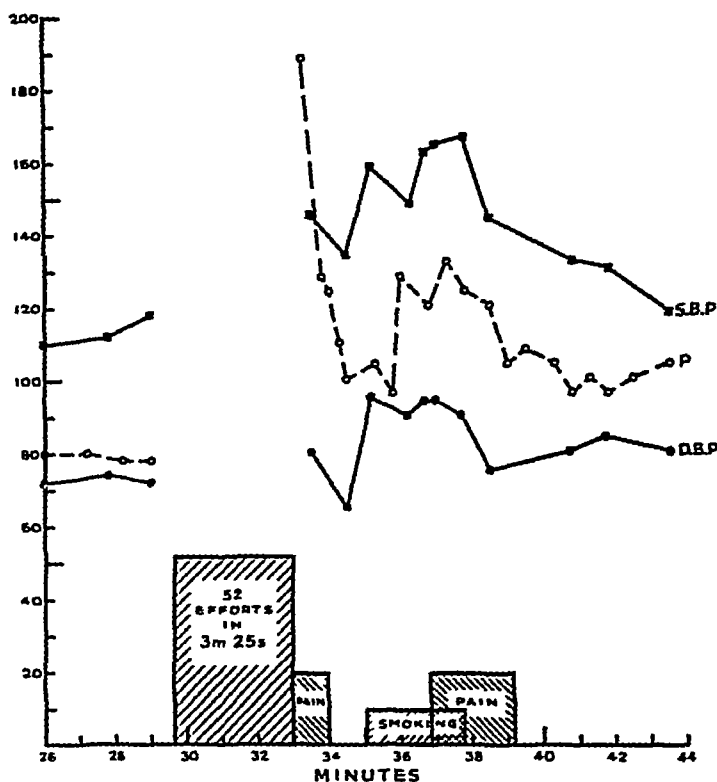
Fig 3   Pain was first produced by exercise   1 minute after pain had disappeared the patient started smoking   Blood pressure and pulse rate rose rapidly and the pain reappeared   Note the rise of pulse rate sustained for 2½ minutes to 120 per minute or over

Smoking at rest   On two occasions after the patient had been at rest for at least 20 min , smoking a cigarette failed to produce pain   The pulse rose to 104 and 112 , and, in contrast to its response to exercise, the diastolic pressure as well as the systolic rose (Fig 2)   Although the patient said he

* One effort here refers to one ascent and descent of the staircase

' did not inhale tobacco smoke, he was observed to do so in all observations here reported

Smoking before and during exercise Immediately after smoking a cigarette, with the effects just described, the patient walked over the staircase 45 times in 3 min 17 sec , before pain began. On another occasion, 2 min after beginning smoking he walked and continued to smoke till pain began after 56 efforts in 4 min 10 sec Not only was the exercise tolerance unimpaired by such smoking, but the duration of pain (75 and 60 sec), and the changes in the pulse rate and blood pressure were essentially similar to those seen after exercise alone
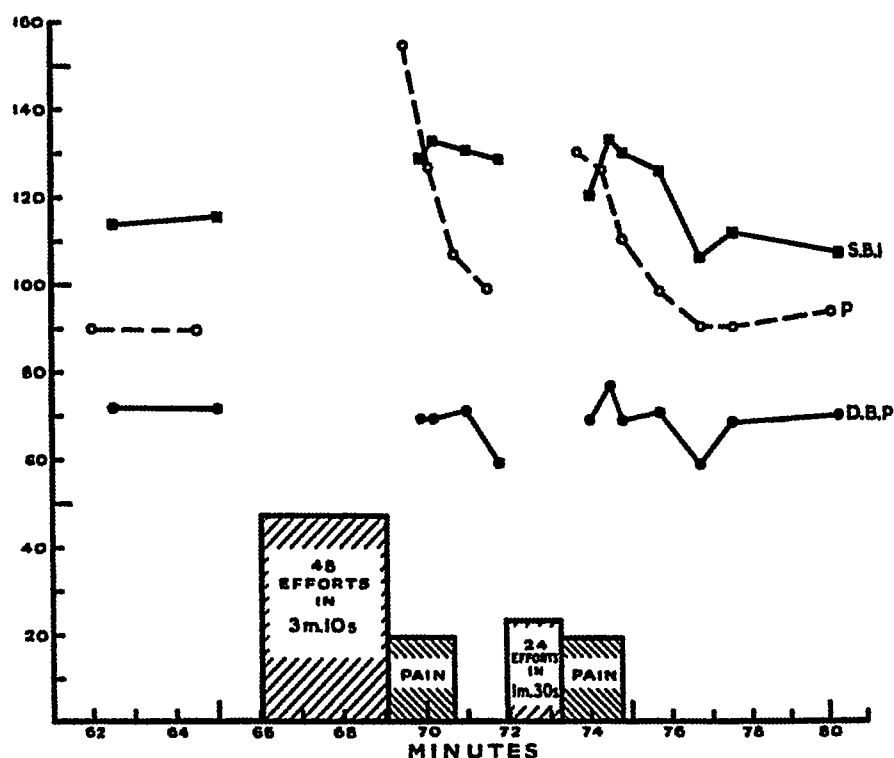
Fig 4 Pain was produced by exercise 1 minute 20 secs after pain had disappeared the patient began to exercise again The exercise tolerance was reduced to half the normal, indicating incomplete recovery of the heart muscle

Smoking after the disappearance of pain The first successful induction of anginal pain by smoking in this patient is illustrated by Fig 3, where a cigarette was begun one minute after the pain induced by effort had disappeared That the heart muscle had not fully recovered from the effects of exercise when smoking was begun is shown in Fig 4, which records an observation made on the same day as that of Fig 3, and in which a second period of exercise was begun 1 minute 20 sec after the disappearance of the pain induced by effort , the number of efforts required to initiate pain was reduced to 24 4 subsequent attempts to induce pain by smoking in th manner illustrated by Fig 3 were unsuccessful, but in all these unsuccessfu

observations the rise of pulse rate was smaller and less sustained Thus in Fig 3 the pulse rate rose to 134 and remained at 120 or over for 2¼ min , in the 4 unsuccessful observations, the pulse only rose above 120 in 2 and then for only 20 sec at most

The effect of atropine It had now become abundantly clear that in this patient smoking would only produce anginal pain if certain conditions were satisfied One of these seemed to be exceeding a certain level of heart rate We therefore repeated the observations after intravenous injection of 1 3 mg atropine sulphate, which Wayne and Laplace (26) found to reduce
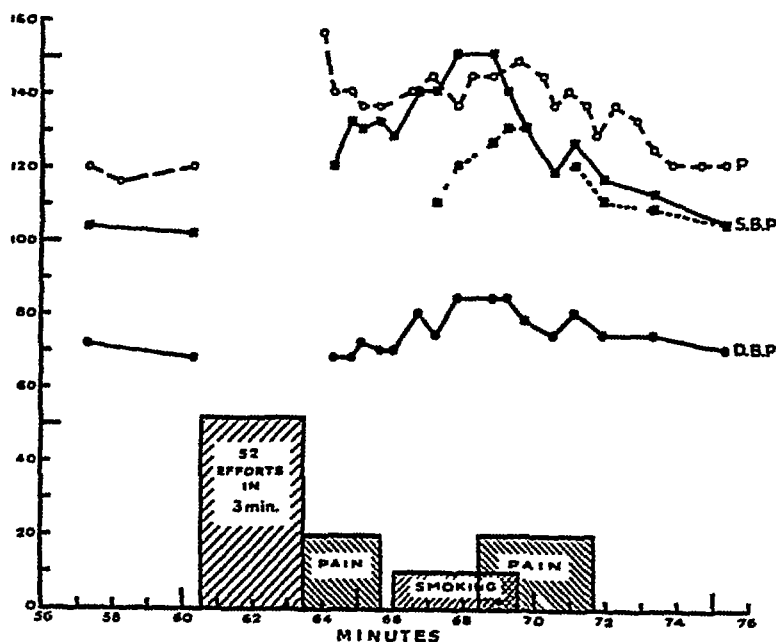
Fig 5 Shows the production of pain by smoking after exercise in the atropinised patient Between the 67th and 75th minute alternation of the pulse was observed the lower systolic level is indicated by the solid squares and interrupted line

the exercise tolerance and prolong the subsequent pain in 4 patients with angina of effort On the two occasions when it was employed, this dose of atropine raised the heart rate in the resting patient from 72 and 70, to 120 and 116 respectively after 30 min After such changes in heart rate had occurred, smoking a cigarette, while still at rest, on 2 occasions failed to produce pain, though the pulse rate rose to 132 and 136, and the blood pressure to 150/96 and 138/84 Twice from rest the atropinised patient walked 44 and 52 times over the steps before pain was induced , the pain lasted 90 and 130 sec after sitting down Our patient thus differed from Wayne and Laplace's patients in not showing a reduction in exercise tolerance

following atropine injection, but he resembled their patients in the prolongation of the subsequent anginal pain   On both of these occasions the patient began smoking 20 and 25 sec after the pain induced by exercise had disappeared , in both the pain reappeared during smoking, to fade after the cigarette was finished   The pain reinduced by smoking lasted 40 and 190 sec and the pulse rate rose to 144 and 148 respectively   In one of these observations (Fig 5) alternation of the pulse was shown by the sphygmomanometer a minute after smoking began and over a minute before pain reappeared Because of this no further tests were made

Comment   It is clear from these observations that smoking was no more than a minor factor in the production of pain in this patient   It would only produce anginal pain, first if it produced a sufficient acceleration of the pulse and second if the heart were in the non-resting state, as exemplified by the period immediately following the end of pain induced by effort , in which period, as Wayne and Graybiel (25) have shown, exercise tolerance is reduced   In this light must be viewed the patient's statement that smoking would induce the pain   For it was clear on questioning him that it was chiefly when he was worried or excited that this effect was noticed, and on some of these occasions he would walk to and fro   Even in these instances, of which the patient was the only witness, and we consider a reliable witness, it is probable then that smoking was not the only factor, though it may have been the precipitating factor, in the production of anginal pain

Discussion

The only hypothesis consistent with the known facts concerning angina pectoris is that which attributes the pain to the accumulation in the heart of a substance or substances liberated from the myocardium during contraction and normally removed by the circulating blood   This hypothesis is based on an analysis of the factors concerned in the production of pain in skeletal muscle, where external work can be measured and blood supply controlled, and upon the clinical resemblances between angina pectoris and intermittent claudication   It is consistent with the known facts concerning myocardial infarction (Lewis, 17) and those concerning angina of effort and the effects on it of drugs (26), food (25), and anaemia (22) , although in the case of the heart neither internal work nor blood flow can be measured In terms of this hypothesis tobacco could contribute to the production of anginal pain either by increasing the work of the heart or by constricting the coronary arteries or by both factors

The very widely held view that tobacco may constrict the coronary arteries seems to have been first advanced by Huchard to explain tobacco angina   Support has seemingly come from observations on man showing that smoking produces vasoconstriction in the limb, though Abramson, Zazeela, and Oppenheimer (1) have shown that this effect is restricted to the

ANGINA AND TOBACCO

skin, and that skeletal muscle blood flow is not reduced   Animal experiments designed to elucidate the action of tobacco on the coronary arteries have yielded conflicting results   Meyer measured the coronary flow in dogs by direct cannulation of the coronary sinus, and observed the effect of numerous drugs   Nicotine in a dose of 10 mg caused an initial fall of blood pressure, without conspicuous alteration in coronary flow, followed by a rise in blood pressure accompanied by a diminution in coronary flow   Laubry, Walser, and Deglaude (15) examined the effect of nicotine and of tobacco extract on the coronary circulation of the perfused rabbit heart, and found the following results at varying concentrations of nicotine   with 0 8 to 2 mg /l , increase of coronary flow from 30 to 60% above resting level   With 4 to 4 8 mg /l , increase of flow, from 5 to 20%   With 8 to 40 mg /l, decrease in flow, from 10 to 20% below resting level   If we assume a blood volume of 2 litres for Meyer's dogs, then the nicotine concentration in them would be of the order of 5 mg /l, which would be expected to cause a slight increase in coronary flow according to the results of Laubry (15)   Uncertainty about the blood levels of nicotine reached in smokers makes these results still more difficult to apply to the problem of tobacco angina

In the patient studied in this paper no evidence was found to suggest that smoking produced coronary vasoconstriction , for smoking before or during exercise did not reduce the exercise tolerance   All the evidence obtained is consistent with the view that in this patient smoking was a factor in producing anginal pain by virtue of its increasing the work of the heart through raising the pulse rate and blood pressure   A similar conclusion has been reached by Graybiel, Starr and White (11) but their evidence has not yet been published   This conception raises a number of problems which require discussion   In the first place many observers have found in a large number of normal and diseased subjects that smoking normally raises blood pressure and pulse rate   The results of Roth, McDonald and Sheard (24) are representative of the effects generally found , they obtained, after 2 " standard " cigarettes, or 2 mg nicotine intravenously, blood pressure increases ranging from 10-35 mm Hg in the systolic and 6-20 mm Hg in the diastolic values, and pulse rate increases of from 20-52 per minute   Control tests in which unlighted cigarettes were puffed, or " corn silk " cigarettes smoked, produced no such changes   On the other hand Johnson (14) found that of 20 individuals, 5 showed no change in blood pressure, and the remainder a fall, the average fall for the whole group of 20 being 4 9 mm systolic and 3 4 mm diastolic   The pulse rates were not mentioned   Nor did he state how rapidly the subjects smoked, nor whether they inhaled or not, important points in attempting to assess the amount of nicotine absorbed. Grollman (12) observed the effect of tobacco on the cardiac output, using the acetylene technique , he found that " usual doses " in habitual smokers had no effect on the blood pressure or cardiac output and caused little or no increase in pulse, but it is not stated whether or not these subjects inhaled " Heavy " smoking, especially of cigars, caused a rise of pulse rate of up to

20 per mm , and a moderate rise in blood pressure and cardiac output In one subject who inhaled, one cigarette raised the pulse from 59 to 66, the blood pressure from 112/68 to 134/88, and the cardiac output from 4 4 l/mm to 5 5 l/mm , but this was regarded as an exceptional response

If this be the common effect of tobacco it may be asked why it is that so few patients with angina of effort have found that pain is precipitated by smoking, for all writers are agreed that the complaint is rare   Individual variations in the degree to which the products of smoking are absorbed, and in the response to these products are no doubt in part responsible   But there is another consideration   It will have been obvious to the reader that in the case fully investigated here considerable difficulty was experienced in finding conditions such that smoking would bring on anginal pain, and it was clear that smoking was no more than a minor factor which, so to speak, would tip the balance in favour of pain   In terms of the hypothesis, it was only when the concentration of the pain factor in the heart was just below the threshold level for pain, that smoking would precipitate an attack It may well have been that this patient was exceptional in having smoked many times in his daily life when his heart was in this condition   In our experiments this condition of the heart was found in the period immediately following the disappearance of pain produced by exercise, when, as Wayne and Graybiel (25) showed, the exercise tolerance is reduced and when, as Lewis, Pickering, and Rothschild (16) showed for skeletal muscle, removal of pain factor from the muscle is incomplete   In his daily life the patient seems to have reproduced these conditions by a combination of excitement, which alone produced pain on one occasion, and slight exercise

A further point requires attention   In Case 2 the patient noticed a reduction in his exercise tolerance during the hour or so following smoking In many of the recorded cases exercise tolerance increased after giving up tobacco and in our third patient, a shrewd observer, this improvement is stated to have occurred over a period of more than 3 months   It is clear that in these cases the effect of tobacco outlasted, and in many cases long outlasted, the period of smoking   The nature of this prolonged action is not evident   In Case 1 the pulse remained faster than normal for 25 mm. after smoking, but the subsequent responses of blood pressure and pulse to exercise were not significantly altered, nor was exercise tolerance increased It is clear that more information is required about patients who show this peculiarity, and particularly of the responses of blood pressure and pulse rate to exercise during the periods before and after smoking has been given up This we have been unable to obtain

Abuse of tobacco is not the cause of coronary sclerosis, the pathological lesion underlying angina pectoris   This was conclusively shown by White and Sharber (27) who found the frequency of non-smokers higher and of heavy smokers lower in 750 cases of angina than in 750 control cases of similar sex and age distribution and drawn from the same walk of life   The present paper by providing an alternative explanation has rendered redundant

the hypothesis that constriction of the coronary arteries by tobacco is the cause of anginal attacks precipitated by smoking, and has provided some evidence against this hypothesis

The retention of the term tobacco angina to describe such cases seems scarcely justifiable, where the rôle of smoking is apparently similar to that of a heavy meal Its use in the second group of cases, mentioned at the beginning of this paper is open to the objection that considerable doubt must remain as to whether the pain is anginal until further facts are available

Summary

1   Three cases of angina of effort are reported in which the patient had noticed that smoking would produce an attack (in two cases) or that the exercise tolerance was influenced by previous smoking (in two cases)

2   In one case the factors concerned in producing anginal pain by smoking have been analysed   It was found that smoking just before or during exercise did not reduce exercise tolerance   Smoking would only produce anginal pain in the period shortly after the disappearance of anginal pain produced by effort, and then only if the pulse rate rose sufficiently

3   In this case therefore smoking was a minor factor in the production of anginal pain and its effect can be explained through its increasing the work of the heart

4   Doubt is expressed as to the usefulness of the term " tobacco angina " A group of cases quite distinct from angina of effort has been described in previous writings, but there is as yet no evidence that in such cases the pain arises from the heart

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This early increase in weight of the shocked kidneys contrasts sharply with the normal weight of kidneys from shocked animals 5 hours after burning (Experiment IV) The organ first becomes heavy due to retention of blood, but the tissues soon begin to lose water and the weight decreases in spite of an increased content of blood. This tissue dehydration can be expected to become progressively greater and may interfere with cellular function Thus, in shock the function of the organs may be impaired not only because of ischæmia, but also because of severe dehydration of the parenchymal cells

It has been suggested that in shock there is a specific nephrotoxic agent There can be no question but that the kidneys are severely injured but the disturbance' responsible for their impaired function is not specific to them since similar changes are found in all organs examined The tests of renal function are more sensitive than those of other organs such as the heart, liver, adrenals, lungs, etc (24) It is likely that if the function of these other organs could be more accurately studied, early and profound changes would be discovered

Experiment X

The toxic factor in extravasative shock

We have previously observed that when an animal is dipped at 75°C for 10 seconds, œdema appears rapidly, but changes little if at all during the last 20 hours (Fig 3, Curve C) In Experiment II it was shown that bleeding volume fell progressively in the last 15 hours of the 24 hour period of observation, during which time only a slight increase in œdema took place Furthermore, it was observed that during this period of time there was a progressive diminution in the hœmoglobin determination (Fig 4) It is, therefore, apparent that there was a reduction in bleeding volume which could not be accounted for by extravasation of the fluid into the burned area, but must represent a new process taking place in these animals This new process is undoubtedly the same as that causing a reduction of bleeding volume in toxic shock because in animals scalded at 75°C up to the head the hæmoglobin content of the liver and kidneys is also increased (Fig 5 and 6), the weights of these organs are altered little if at all (Fig 5 and 6), and the microscopical changes are similar to those found in toxic shock, i e , congestion and increase in the number of open capillaries

Scalding a large area of the body surface (90%) at 65°C for 10 sec seems to provide the most ideal circumstances for the production of local fluid loss in the burned areas Like Elman (6), we have observed that at higher temperatures, less œdema occurs, and that at lower temperatures, less shock and less œdema occur Yet, under these circumstances (65°C for 10 sec), ideal for the production of œdema, it can be stated that the toxic factor causes further accentuation of the shock and later plays the predominant role in the deleterious state of the animal

Experiment XI

Demonstration of humoral factor   -

The demonstration of the humoral factor in shock is fraught with difficulties, and indeed no convincing demonstration of such a factor has been presented   Harkins (12), mentions 20 toxic substances which have been proposed, but none of these has been generally accepted as the cause of shock

In order to demonstrate a burn toxin the following points are important   1   The blood should contain the largest possible quantity of the toxic agent   2   Extremely large amounts of this blood should be used   3   Since the chemical nature of the toxic agent is unknown, it is inadvisable to extract the blood, since one does not know what to extract   Therefore, the blood should be rapidly drawn from the shocked animals, heparinized, and injected as rapidly as possible into the recipient   4   The recipients of the blood should develop the essential disturbance of toxic burn shock, namely, a diminution of effective circulation   The recipients need not have the clinical manifestations of shock because even under these ideal circumstances, the amount of toxic substance injected may be insufficient to cause them   It should be remembered that when one injects blood from a burned animal, not only is the toxic agent given, but the whole blood has beneficial factors which can be expected to compensate partially for the toxic substance contained in the same blood   Thus, we have repeatedly observed that the injection of large amounts of whole blood from severely burned animals does not have a clinically deleterious effect on normal animals

It was previously shown that animals die in typical shock following a very severe burn (100°C for 20 sec up to the head) in a very short period of time   If there is a humoral factor causing shock, this factor should be very highly concentrated in the blood of these animals   Therefore, we used these rats as donors, bled them from the abdominal aorta or vena cava 5 to 10 min after the burn, heparinized the blood immediately, and injected a volume equivalent to 4% of the body weight intravenously into the femoral vein of normal rats   Equal quantities of heparinized blood from non-burned animals were injected into control animals   Because of the small amounts of blood which could be recovered from shocked rats, it was necessary to bleed 2 or 3 animals to obtain enough blood for one injection   Ten to fifteen minutes after the injection, the bleeding volume of each group was measured

Thirty-eight pairs of rats were compared, after receiving blood from approximately 50 normal rats and 150 burned animals   The results on bleeding volumes are shown in Fig 10   The average bleeding volumes (as % of the original body weight) of 38 normal rats receiving 4% of the body weight of blood from normal animals was 6 7 (standard deviation of a single observation ± 0 63 and standard error of the mean, ± 0 10) while an equal number of similar rats receiving blood from burned rats was 5 7 (standard deviation of a single observation ± 0 83 and the standard error

of the mean, $\pm$ 0 14) The t value for the deviation of these means is 5 8 so that the P value is less than 0 01 These results appear to be significant The difference between the effect of blood from normal and shocked animals is more striking when calculated in the following manner The bleeding volume of normal rats averages 4 1% of the body weight (Experiment VII) After receiving 4% of normal blood, the bleeding volume is 6 7%, a difference of
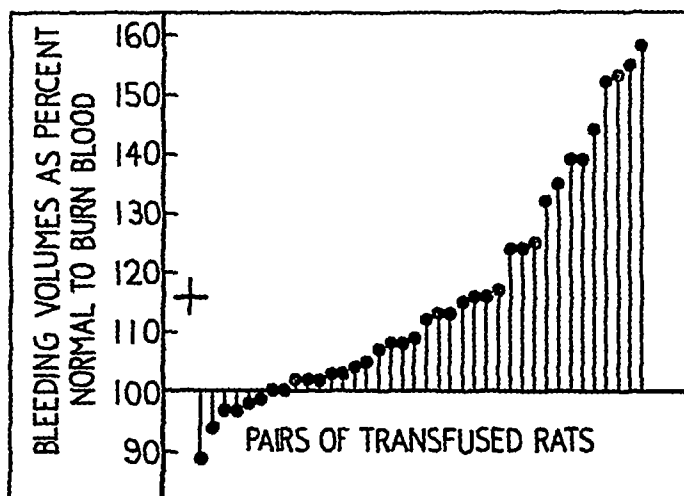

Fig 10 Each dot represents the ratio of the bleeding volume of a rat transfused with blood from normal animals to the bleeding volume of a similar rat transfused with blood from severely burned animals Two similar normal rats were injected at the same time, one with blood from normal rats the other with blood taken from rats 5 minutes after scalding to the head at 100 C for 20 sec The blood was heparinized and administered intravenously, 4% of the original body weight within 10 to 30 min after collection After 10 min the bleeding volume of each pair was determined The ratio of the bleeding volumes normal to burn blood response should fall on the 100 line if there were no difference When the dot is above the 100 line the bleeding volume of a rat receiving blood from burned rats is less than that of its control receiving blood from normal rats The reverse is true for dots below the 100 line The length of each line represents the magnitude of the difference in response The cross represents the mean of all the values the vertical line of the cross being the spread of the standard error of the mean. There appears to be a significantly decreased bleeding volume in animals receiving blood from burned rats as compared to animals transfused with normal blood

6% The bleeding volumes of animals receiving blood from burned nimals was 5 7%, a difference of 1 6% Hence, 62% less blood was covered from animals receiving blood from shocked animals

This experiment demonstrates that there is a factor in the blood of rned animals which causes a diminution in the effective circulation As entioned above, this experiment does not prove that a new substance, nic in origin, is absorbed from the burned area might absorb some

substance from the blood which is necessary for normal capillary function
The only way of differentiating between these two possibilities would be
by the concentration or isolation of the toxic substance from the blood of
burned animals   The injection of a small volume of a substance so obtained
causing the characteristic phenomena here described would conclusively
prove that such a substance is the cause of shock, providing it is not present
in normal blood   Such evidence, however, is not available and the second
possibility, namely, quantitative chemical change in the blood, cannot be
eliminated

The only substance demonstrated so far which accumulates in unusually
large quantities in the burned area is the sodium ion (7)   Although a
reduction in the blood sodium (14) has been found in the burned animal,
this reduction would have to be present immediately (1 min) after the burn
if a change in the sodium ion concentration is the cause of the capillary
atony

DISCUSSION

A series of experiments has been performed to elucidate the nature
of burn shock   It has been shown that the primary change is a humoral
one which causes dilatation and an increase in the number of open capillaries
of the viscera   This, in turn, causes the stagnation of blood, especially red
cells, with resulting decrease in the bleeding volume and in the effective
circulation   The most important factor regulating capillary tone seems to
be the metabolic needs of the tissues   Such factors as the pituitary and
adrenal hormones and the sympathetic nervous system seem to be of
secondary importance, for animals do not immediately die in shock due to
opening up of the capillaries following removal of the adrenal medulla, the
pituitary, or the sympathetic nerves   In burn shock, a humoral change
takes place which causes capillary dilatation and an increase in the number
of open capillaries considerably above the metabolic needs of the tissues
Indeed, as a result of these capillary disturbances, a paradox occurs   As a
result of capillary dilation, the bloodflow is decreased instead of increased

The theory of capillary congestion and arterial constriction was first
suggested in 1879 by Mapother (15)   Many investigators, especially Moon
(16) and Cannon (4), have been ardent supporters of this theory   These
two men have obtained a great deal of evidence in favour of the toxic theory
of shock, and have held firmly to their viewpoints in the face of strong opposi-
tion   It would be well to see how far the criticisms of this theory apply
to the findings obtained in this group of experiments

1   It has been claimed that local fluid loss was sufficient to account
for shock after burns   This objection is not valid because we have previously
shown that in toxic burn shock there is little or no fluid loss (19), and that in
extravasative burn shock the toxic factor plays an important role
(Experiment X)

2 It has been stated that congestion is not always present   This statement would appear to be based on a lack of quantitative and objective observations   For, if the amount of blood in the shocked organ is determined by weight (Experiment IX), the red blood cell content determined by colorimetric methods (Experiment IV), and the capillaries carefully observed, counted, and measured (Experiment VI), there can be no doubt that visceral congestion and capillary atony are present

3 It has been stated that the degree of the capillary congestion has not been measured and might not be of sufficient magnitude to accommodate large volumes of blood   By quantitative studies we have shown that this is not true (Experiments IV, VI, IX)

4 It has been stated that the capillary congestion is a terminal event and due to anoxia   This is not true for we have shown that capillary atony occurs immediately following the burn and in 1 minute the tissues show evidence of marked congestion with resulting diminution of bleeding volume (Experiment VIII and IX)   The anoxia does not cause the capillary congestion but is a result of the circulatory disturbances caused by the trapping of the blood in the small vessels

5 It has been suggested that the heart may be damaged in shock and that the capillary congestion is due to heart failure   This is not true for the following reasons   (a) The hearts of shocked animals after exsanguination from the abdominal aorta continue to beat after the venous return has virtually ceased   The failure of the venous return cannot be due to myocardial weakness for the heart still beats and the blood is trapped in the periphery (Experiment II)   (b) The capillary congestion occurs immediately after the injury, before the heart would have a chance to fail (Experiment VIII and IX)   (c) Shocked animals without hearts have as marked capillary congestion as do shocked animals with hearts (Experiment VII)

6 It has been claimed that a humoral change which can account for the shock syndrome has not been adequately demonstrated   This is no longer true for the humoral factor has been demonstrated (Experiment XI)

In this study we have only considered the *sequence of events in the early stages* of burn shock   It is possible, and indeed likely, that the process later becomes more complicated as a result of generalised ischæmia, and perhaps infection   Infection would appear to play no role during the first 24 hours in our experiments, for the administration of sulphamerazine had no influence on survival time nor mortality in mice (2)

These observations are in marked contrast to the shock which results from the crushing of large amounts of muscle in dogs (20), where the administration of sulphamerazine completely prevented the onset of shock, proving the bacterial origin of this toxic factor This shows how complex the etiology of shock is for it has now been demonstrated that there are at least two " toxic " factors, bacterial, and non-bacterial These observations show that shock is not a distinct entity but results from a variety of causes and mechanisms

SUMMARY

1 Denervation by severing the spinal cord has no effect on the mortality or the survival time of rats burned at 100°C for 15 sec Since it has been previously shown that in this type of burn there is insufficient local fluid loss to account for the shock-like state, it has been concluded that the shock is toxic (humoral) in origin

2 It has been shown that the bleeding volume in " toxic " and " extravasative " burn shock is reduced, the reduction being proportionate to the degree of trauma and becoming progressively more severe until the animal dies Following less severe burns, after the initial reduction the bleeding volume increases as the animal recovers

3 In toxic burn shock there is no significant elevation of the haemoglobin Conspicuous haemo-concentration occurs only following burns with much local fluid loss It is, therefore, concluded that the degree of haemo-concentration is not indicative of the severity of the burn or of the degree of shock

4 As shock progresses, the haemoglobin values fall In toxic burn shock, anaemia may occur This is partly due to transfer of fluid from the tissues into the blood vessels, and probably to greater retention of the red blood cells than of plasma in the capillaries

5 By histological study, it has been shown that in burn shock there is an increase in the number of open capillaries and in the amount of blood retained in each capillary

6 The capillary atony, visceral congestion, and diminution of bleeding volume occur quickly (within 1 min) after severe burns This is, therefore, the primary disturbance and is not secondary to anoxia, nor is it terminal

7 The visceral congestion present in shocked animals can best be demonstrated after exsanguination The organs of normal animals become very pale, whereas those of shocked animals retain their congested appearance due to the vascular disturbance which prevents vascular contraction

8   The bleeding volume of animals without hearts is approximately the same as those with hearts   This demonstrates the active participation of the peripheral circulation in the venous return   In shock, this peripheral contractile power is impaired so that the venous return is greatly reduced

9   In shock with a considerable degree of local fluid loss, the toxic vascular factor soon becomes operative and after a few hours is the most important cause of the circulatory disturbance

10   A humoral agent in the blood of burned animals can be demonstrated by the reduction of the bleeding volume of normal animals after they have been injected with large amounts of blood from burned animals

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THE RÔLE OF THE KIDNEY IN ACUTE AND CHRONIC HYPERTENSION FOLLOWING RENAL ARTERY CONSTRICTION IN THE RABBIT

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In the course of some experiments carried out in 1938 with Dr Kelsall, it was noticed that in two rabbits with hypertension of some weeks duration, and produced by constricting one renal artery after the other kidney had been removed, excising the sole ischæmic kidney was not followed by a return of the arterial pressure to normal   Subsequently it was found in rabbits that hypertension, similarly induced but of only a few days duration, was abolished by removing the affected kidney   These observations seemed to be important from the point of view of the mechanism of this hypertension, suggesting as they did that the role of the kidney differed in acute and chronic hypertension   Further experiments were made to verify and elucidate these chance findings, and are recorded in this paper

The effect of excising the sole ischæmic kidney on hypertension of short and long duration

Rabbits fed on a mixed diet, including green stuff, have been used throughout, arterial pressure being measured in the central artery of the ear of the warm unanæsthetised animal by Grant and Rothschild's capsule (9)   After a preliminary period of observation, the right kidney was removed, and two weeks later the left renal artery constricted by a clamp of 0 5 mm internal diameter, both operations being carried out with aseptic precautions, through loin incisions, under nembutal anæsthesia   A few animals were lost through partial infarction of the kidney   The remainder developed hypertension, which characteristically was progressive, the arterial pressure rising usually by not more than 20 mm in the first 24 hours, and continuing to rise, at a variable rate and for a variable time, subsequently   The remaining left ischæmic kidney was removed aseptically under ether

TABLE I

Showing the effect of removing the sole ischæmic kidney on hypertension of short and long duration in the rabbit

Rabbit	Duration ischæmia	Arterial Pressure (mm Hg)										Pre operative pressure reached hr after nephrectomy	Death hours	Carcase wt Kg	Heart ×100 / Carcase
		Average pre operative	After ischæmia Highest	After ischæmia Final	Hours after nephrectomy 3–6	6–12	12–24	24–36	36–48	48–72				
Group I		Kidney removed 4 to 8 days after constricting its artery												
244	4d	82	120	120	99	87	85	—	—	84	6½	74	1 3	—
246	4d	81	120	120	95	95	88	80	—	84	0–24	76	1 4	—
200	6d	81	96	96*	70	75	73	70	78	—	2½	40	1 6	—
201	6d	80	98	90*	86	78	82	82	—	77	¾	72	1 6	—
221	6d	82	127	123	75	—	—	50	—	—	4	30	—	—
232	7d	82	113	110	78	—	92	78	82	81	—	80	1 9	0 52
185	8d	89	126	123*	84	—	83	97	99	—	1¾	50	1 20	0 42
1C	7d	71	100	98	100	100	85	64	70	81	—	—	1 8	0 45
2C	7d	68	108	108	77	70	95	80	80	—	5	76K	1 57	0 44
7C	6d	76	108	96	72	74	60	80	61	80	1¼	49	1 44	
8C	6d	82	100	91	68	65	66	70			1½			
Group II		Kidney removed 7 to 15 weeks after constricting its artery												
162	7 wk	78	125	118	103	—	—	117	113	120	3	75	1 0	0 5
150	9 wk	70	105	100	80	—	—	89	—	110	—	60	1 05	0 07
4B	9 wk	50	132	131	132	138	132	126	—	140	—	54	1 1	0 84
5B	9 wk	69	137	133	117	113	107	113	110	115	—	72K	1 3	0 63
6B	9 wk	66	120	105	92	88	91	96	148	98	—	72K	1 2	0 6
8B	9 wk	63	132	101	103	97	123	140	99	142	—	66	1 8	0 81
4A	12 wk	72	110	95	91	97	96	97	92	110	—	72K	1 8	0 43
5A	15 wk	73	120	97	99	101	86	90	—	106	—	72K		0 54
Group III		Renal artery not constricted No hypertension												
231	None	87	—	—	81	—	70	—	73	—	—	60	—	—
233	None	87	—	—	74	—	98	—	78	85	—	50	—	—
243	None	75	—	—	84	80	80	—	—	72	—	70	—	—
117	None	81	—	—	—	—	83	—	08	56	—	71	—	—
128	None	77	—	—	—	—	79	—	61	75	—	72	—	—
132	None	78	—	—	—	—	72	—	78	—	—	72	—	—
134	None	75	—	—	—	—	56	—	61	—	—	50	—	—

* Kidney exteriorised when renal artery clamped

anæsthesia either 4-8 days, or 7-15 weeks after constricting its renal artery, and the arterial pressure recorded at frequent intervals until the animal died or was killed

The results which have, in the main, confirmed our original observations are summarised in Table I   The first series consists of nine rabbits in which the ischæmic kidney was removed by re-opening the loin incision 4-8 days after constricting the renal artery , in three of these rabbits (185, 200 and 201), the kidney had been brought through the muscle layer and made subcutaneous when the renal artery was clamped   The time from induction of ether anæsthesia to the end of the operation varied from 5 to 20 min and, ether being discontinued when the renal pedicle had been tied, the animal was usually able to sit up within 5 min   of completing the operation   In 7
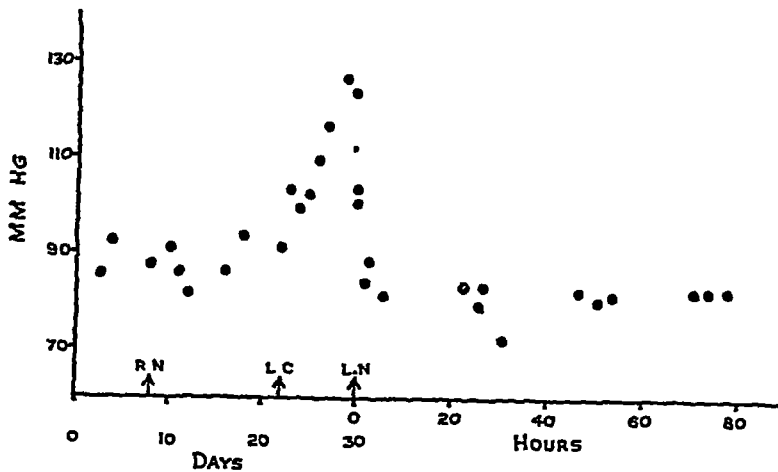
Fig 1   Rabbit 185   Right kidney removed (R.N) 17 10 38   Left renal artery clamped and kidney made subcutaneous (L C) 31 10 38   Left kidney removed (L.N) 8 11 38   Ordinate arterial pressure   Abscissa   time in days to left nephrectomy then in hours   Note that the arterial pressure returns to normal after the ischæmic kidney is excised.

of these rabbits, the arterial pressure fell progressively after removing the kidney until the normal level for the animal (that is to say within 5 mm of the average pre-operative pressure) had been regained, the shortest time for this process being 45 min in rabbit 201, which had the smallest hypertension, and the longest between 6 to 24 hr in rabbit 246 which had one of the largest rises of pressure   Subsequently the level of arterial pressure remained essentially unchanged until within a few hours of death when it was sometimes observed to fall rapidly and profoundly   Fig 1 is representative of the course of the arterial pressure in these animals   In rabbit 232, the arterial pressure fell to the pre-operative level in 30 min   but readings at 19, 20, 21 and 23 hours after nephrectomy were some 10 mm above this , later readings were not made   Rabbit 1c was exceptional in that the arterial pressure remained significantly above its resting level until the last

reading was made 50 hours after nephrectomy , except between 12 and 24 hours the pressure was indeed within a few mm of the highest recorded before the ischæmic kidney was removed

The second series consists of eight rabbits in which the ischæmic kidney was removed 7-15 weeks after the renal artery had been constricted   In these rabbits the nephrectomy was carried out under ether anæsthesia by the transperitoneal route through an anterior abdominal incision, the fibrosis around the kidney making the loin approach more difficult   The operation lasted a little longer than in the preceding series, usually 20-30 min   Nevertheless the animal had often recovered from the anæsthetic within 20 min of tying the renal pedicle, and blood pressures were obtained
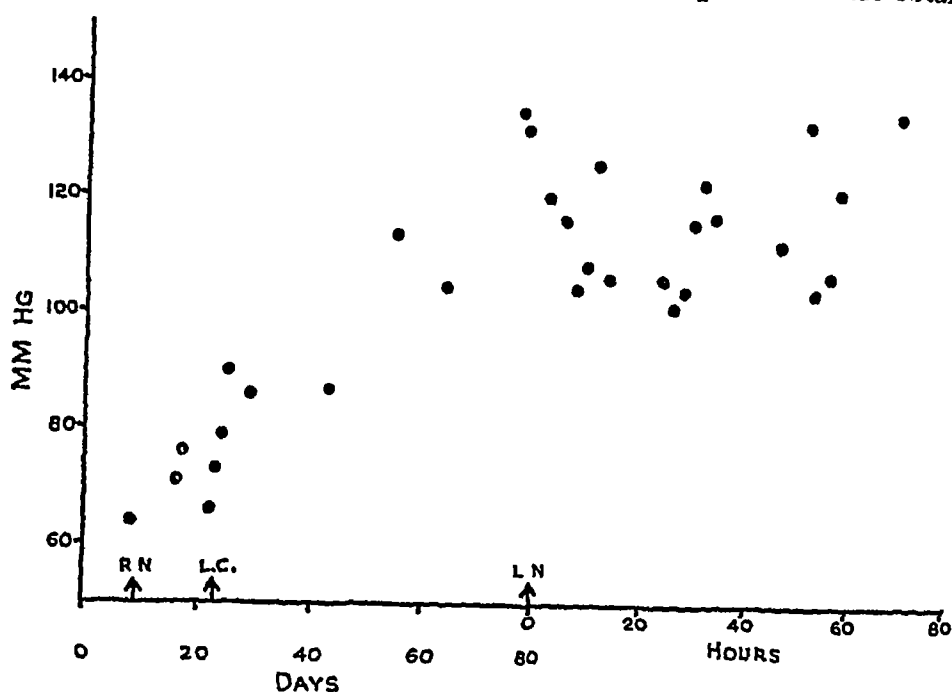
Fig   2   Rabbit 5B   Right nephrectomy (R N) 28 7 43   Left renal artery constricted (L C)   11 8 43   Left kidney removed (L N) 7 10 43   Ordinate and abscissa as in Fig 1   Note the failure of the arterial pressure to return to normal after excising the ischæmic kidney

after 40 min in some animals   In only one of these animals did the arterial pressure return to normal, and this fall, recorded 3 hours after nephrectomy, was transient, the pressure on the third post-operative day having risen to a level higher than the highest reached before the kidney was removed   In most of the remainder there was a tendency for the pressure to fall a little during the first 24 hours, and subsequently to return towards the values recorded just before nephrectomy, but in all the pressure remained well above the normal level   Indeed in rabbit 4B, in which 11 readings of arterial pressure were obtained in the 50 hours after removing the ischæmic kidney, the pressure remained 65 mm Hg , or more above the normal, and never fell more than 10 mm Hg below the highest value reached with the ischæmic kidney still in situ   Fig 2 is representative of this series

The third series in Table I consists of seven rabbits in which the right and the left kidneys were removed at separate operations but in which the renal artery was not clamped and no hypertension developed. In 231, 233, 243, designed as controls for the first series, the left renal artery was exposed but not clamped, 7d 7d and 4d before the final left nephrectomy In 128 the interval between the two nephrectomies was 16 weeks, resembling in this respect animals of the 2nd series Although pressures were recorded less frequently in these animals, it is clear that removing the second kidney left the arterial pressure essentially unaltered until the final fall a few hours before death

It is clear from these results that when in the rabbit the sole and ischæmic kidney is removed, the effect on hypertension depends on its duration If the hypertension is of relatively short duration, up to 8 days, then nephrectomy usually restores the arterial pressure to normal within a few hours and the pressure subsequently remains normal until within a few hours of death Evidently here hypertension is dependant on the presence in the body of the kidney whose renal artery has been constricted On the other hand when hypertension has lasted more than 7 weeks removing the ischæmic kidney does not abolish the hypertension, which usually remains relatively unchanged during the few days in which the animal survives

As has already been indicated excising the ischæmic kidney was technically easier in the rabbits with recent than in those with long standing hypertension The fall in arterial pressure in the first as opposed to the second series cannot therefore be attributed to a more severe post-operative reaction Nor can the differences be attributed to the time of survival after removing the remaining kidney, 5 animals in each group living 72 hours or more The ageing of the animals in the second group may be dismissed as a factor, because the duration of the hypertension was a small fraction of the life-span of the rabbit, and two unoperated litter mates of 4B, 5B, 6B, and 8B showed no rise of pressure during the period of the observation The gradual accumulation of renally excreted substance or substances during the more prolonged period of renal artery constriction is unlikely to have been a factor in the failure of the arterial pressure to fall after nephrectomy in the second series, since in 6B and 8B the blood urea at the time of nephrectomy was respectively 51, and 34 mg per 100 c c The conclusion is therefore reached that the difference in the effect of excising the sole ischæmic kidney in the two series is due to the difference in the time during which the ischæmic kidney had been present in the body and in the duration of the associated hypertension

The effects of nephrectomy on hypertension of short duration in the rabbit are entirely consistent with the hypothesis that the hypertension is due to the release of a humoral agent from the ischæmic kidney, they are in line with the results obtained in the dog in which conclusive evidence for such a mechanism has been obtained All the available evidence suggests

that the agent concerned is renin   In an earlier paper (11) it was shown
that the time taken for arterial pressure to return to normal, after stopping
an intravenous infusion of renin lasting four hours, was similar to the time
taken for the hypertension to disappear when an ischæmic kidney was
removed 4-6 days after constricting the renal artery, a result suggesting
that in hypertension of short duration the period elapsing between removing
the ischæmic kidney and the return of the arterial pressure to normal
represents the time taken for the body to inactivate renin, or the substances
to which it gives rise   It seemed possible therefore that the failure of the
arterial pressure to return to normal after nephrectomy in the rabbit with
long continued hypertension might be due to some slowing in the process
of inactivating renin   To investigate this possibility the responses to renin
were tested in two such animals after excising the ischæmic kidney   Highly
abnormal responses were observed   In order to place these responses in
their perspective it seemed proper to investigate the part played by
nephrectomy and hypertension in their causation

The effect of nephrectomy and of hypertension on the response to renin

The response to renin is similar in kind but varies in size and duration
in different normal rabbits   The effects of nephrectomy and of the various
stages of hypertension have therefore been investigated by comparing the
responses to renin of animals subjected to these procedures with the responses
given to the same dose and preparation of renin by the same animal before
any operation   Unless stated otherwise the renin used was prepared by
extracting an alcohol dried rabbits kidney powder with 10 c c saline per g ,
this powder remains stable for years (20)   The same powder was used
throughout the experiments on any one animal, thus ensuring comparable
stimuli   Injections were given into the vein of an ear rendered insensitive
a few minutes previously by injecting 0 25 c c 1 % procaine around the main
nerves at its base   In all cases the animal was unanæsthetised, the arterial
pressure being recorded on the other ear by the capsule method   Where
more than one injection was given on one day, and this was exceptional,
an interval of some hours elapsed between injections

The effect of nephrectomy and ureteric ligature in the normal rabbit

The effect of removing both kidneys on the response of the otherwise
normal rabbit is summarised in Table II   The figures for each rabbit
represent the mean of at least two experiments carried out 4-6 days before
and at least two experiments 24-72 hours after removing the second kidney
In each rabbit the response to renin is increased by excising all renal tissue,
both at the peak of the rise (2 min) and at comparable times during its fall,
so that the duration of the response is increased, but it may be noted in each
rabbit the initial pressure in the nephrectomised period was lower   Fig 3,
which is representative, demonstrates the increased height and duration

TABLE II

The influence of bilateral nephrectomy and bilateral ligature of the ureters on the response of the rabbit to a single injection of renin

Rabbit	B P before operation mm Hg				B.P after operation mm Hg			
	Initial	Rise to renin			Initial	Rise to renin		
		at 2 min	at 10 15 mm	at 25 30 min.		at 2 min.	at 10 15 mm	at 25 30 mm
A. Bilateral Nephrectomy								
117	81	30	10	4	75	44	17	11
128	77	26	6	0	65	34	11	5
132	78	43	19	7	75	62	24	19
134	75	43	11	2	58	73	28	22
Average	77	35	12	3	69	53	20	14
B Bilateral ligature of ureters								
141	74	35	13	7	77	42	9	6
142	71	28	15	5	87	25	7	6
143	69	30	15	2	71	43	18	18
Average	71	31	15	4	78	37	11	10

of the response after nephrectomy , it also suggests what is already apparent from inspection of the Table, namely, that part of the difference in the responses is the result of the lower initial pressure after nephrectomy For comparison, the responses obtained 1 to 7 days before ligature of the first and 20 to 72 hours after ligature of the second ureter, are shown. Here the effects are less striking and less regular , there is no lowering of the initial pressure after ligature of the ureters, and no constant increase in the size or duration of the response to renin While these results indicate that nephrectomy increases the height and duration of the response to renin, our results with ureteric ligatures are insufficient to show whether this change is due to the removal of renal tissue or to uræmia, though they favour the first of these two alternatives

That nephrectomy increases the height and duration of the pressor response to renin was first observed by Tigerstedt and Bergman (24) in the rabbit and has been confirmed for this animal and the dog The effect

has been interpreted as evidence that the kidney is concerned in inactivating renin or its product hypertensin, but Houssay and Dexter (13) observed that the increased response in the dog was less pronounced after 3 than it was 48 hours after nephrectomy, at 48 hours increased responses were obtained to hypertensin and adrenaline also   Dexter (4) has found that in the dog nephrectomy does not increase the plasma hypertensinase   Houssay, Braun-Menendez and Dexter (12) have found that the time during which injected renin can be detected in the circulating blood of the dog under chloralose anæsthesia is prolonged from the normal 30 min to rather less
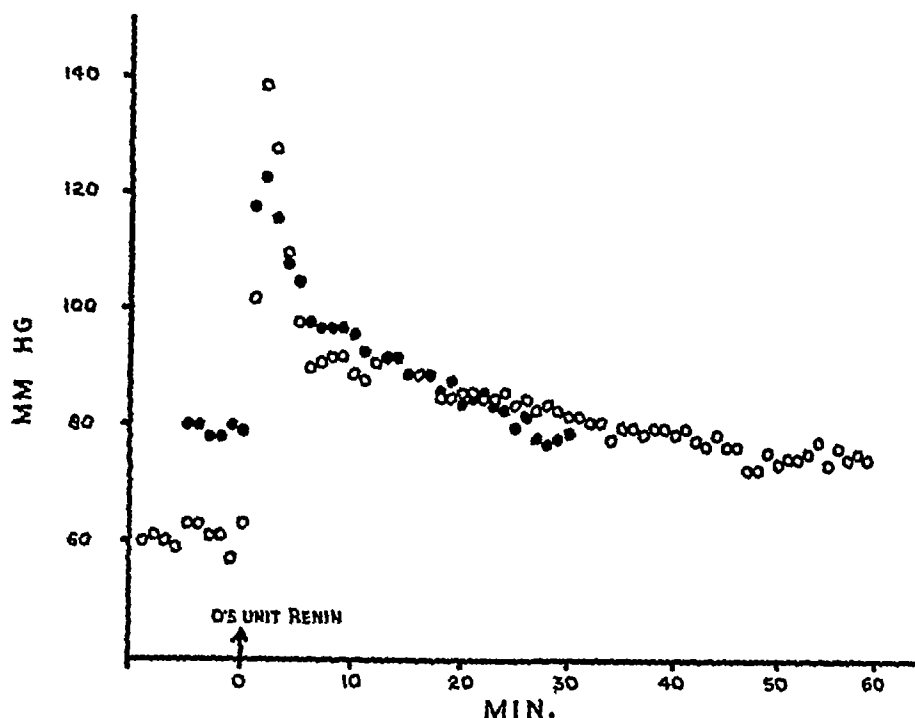
Fig 1   Black discs represent the readings of arterial pressure of a normal rabbit, 134, before and after injection 0 5 unit renin on 10 11 37   Open circles represent the response of the same rabbit to the same dose of renin on 16 11 37, 24 hours after removing the left kidney, the right having been removed on 11 11 37

than 2 hours just after nephrectomy, and to 3-4 hours 49 hours after this operation   They considered that this progressive increase in the survival of injected renin in the hours after nephrectomy might indicate either that renin was destroyed by a substance secreted by the kidney which persisted in the circulation some time after nephrectomy, or that renin was destroyed by some tissue metabolic process which suffered in uræmia   Finding that replacement of 87 5% of the blood volume with normal blood did not reduce the survival time of renin in uræmic animals, they concluded that the second alternative was correct   Govaerts (8) has shown that interposing a normal kidney between carotid artery and jugular vein raises the arterial pressure of the recipient dog only slightly 2 hours after both its kidneys have been

TABLE III

Comparing the responses to renin in unanaesthetized rabbit during the course of hypertension produced by constricting the artery to the sole remaining kidney and after removing the ischemic kidney   III arterial pressure (B P) in mm Hg   Duration of renin response in min

Rabbit	Before hypertension				1st week after renal artery constriction				Ninth week after renal artery constriction				After removal of ischemic kidney				
	Dose mm	Initial BP	Rise BP	Dura tion	Dose mm	Initial BP	Rise BP	Dura tion	Dose mm	Initial BP	Rise BP	Dura tion	Dose mm	Initial BP	Rise BP	Dura tion	Hours after nephrectomy
Group I	Sole kidney removed 0 to 7 days after constricting its artery																
1C	0 5 / 0 5	71 / 71	28 / 15	17 / 17	0 5 / 0 5	98 / 95	11 / 28	10 / 10	— / —	— / —	— / —	— / —	0 5 / —	109 / —	40 / —	— / —	42 / —
2C	0 5 / —	73 / —	21 / —	20 / —	0 5 / 0 5	105 / 102	8 / 18	15 / —	— / —	— / —	— / —	— / —	0 5 / —	70 / —	40 / —	60 / —	48 / —
4C	0 5 / —	75 / —	11 / —	60 / —	0 5 / 0 5	102 / 101	14 / 15	11 / 20	— / —	— / —	— / —	— / —	— / —	— / —	— / —	— / —	— / —
7C	0 5 / 0 5	86 / 87	21 / 28	— / —	0 5 / 0 5	106 / 98	10 / 35	— / —	— / —	112 / 111	11 / 11	— / —	0 5 / 0 5	82 / 82	17 / 10	33 / 11	27 / 46
8C	1 0 / —	78 / 82	40 / 71	— / —	1 0 / 1 0	90 / 91	45 / 17	11 / —	— / —	95 / 101	— / —	— / —	1 0 / —	71 / —	11 / —	02+ / —	20 / —
Group II	Sole kidney removed 0 to 15 weeks after constricting its artery																
4A	— / —	— / —	— / —	— / —	— / —	— / —	— / —	— / —	— / —	— / —	— / —	— / —	1 0 / —	91 / —	50 / —	215+ / —	27 / —
5A	— / —	— / —	— / —	— / —	0 5 / —	82 / —	15 / —	20 / —	— / —	— / —	— / —	20 / 42	1 0 / —	80 / —	50 / —	102 / —	20 / —
1B	— / —	77 / —	25 / —	— / —	0 5 / 0 5	70 / 82	23 / 27	21 / —	0 5 / 0 5	121 / 131	30 / 11	12 / —	0 5 / —	120 / —	42 / —	120 / —	17 / —
6B	0 5 / —	71 / —	11 / —	21 / —	0 5 / —	81 / —	21 / —	15+ / —	0 5 / —	95 / 101	55 / 58	63 / 20	0 5 / —	121 / —	72 / —	80 / —	51 / —
4B	0 5 / —	65 / —	21 / —	— / —	0 5 / —	81 / —	20 / —	20 / —	0 5 / —	95 / 101	— / —	— / —	0 5 / —	110 / —	05 / —	80 / —	17 / —
8B	1 0 / —	65 / —	71 / —	60 / —	1 0 / —	81 / —	26 / —	20 / —	0 5 / —	107 / —	11 / —	60 / —	0 5 / —	118 / —	11 / —	— / —	17 / —

removed, but conspicuously 2 days later , by crossed transfusion experiments
he has demonstrated that this phenomenon is due to a change in the
sensitivity of the nephrectomised recipient to the pressor substance leaving
the perfused kidney

The effect of hypertension   Table III summarises the responses to renin
during the first and ninth weeks of hypertension following arterial
constriction of the sole kidney   During the first week the response is some-
times decreased, sometimes increased and sometimes unaltered   Closer
inspection of the figures suggests that the effect is dependent on the degree of
hypertension , for the response was diminished in 1C, 2C and 4C in which
the rise of pressure due to renal artery constriction exceeded 25 mm Hg ,
while the response was essentially unaltered or increased in the remainder
in which the hypertension was less   If discharge of renin by the ischæmic
kidney is the sole factor producing hypertension during the first week, then
a decrease in the response to injected renin is to be anticipated , in a previous
paper it was shown that equal increments in dosage of injected renin are
accompanied by progressively smaller increments of response (18) , and
Taggart and Drury (23) have shown that the response to a single injection
of renin is reduced when the arterial pressure has previously been raised by a
continuous infusion of the substance   While the diminished response in the
three animals with the more severe hypertension may be viewed in this light,
the results are too few and too variable for detailed comment

Responses to renin during the ninth week after renal artery constriction
were greater and lasted longer than those in the first week in all four animals
in which the comparison was made   In the 3 animals where results are
available, the response in the ninth week was also greater than it had been
before the renal artery was constricted (in 8B the dose injected in the ninth
week was half as great as that injected in the previous two periods)   These
findings of an increased response to a moderate dose of renin are in agreement
with those of Kapp, Friedland and Landis (14) , Taggart and Drury (23)
found rather similar responses in normal animals and animals with
hypertension, though they did not test the same animal before and after
hypertension as has been done here

It is possible that this increase in response to renin in developed
hypertension is part of a more general responsiveness of the vessels to pressor
substances for increased responses to adrenaline, tyramine and posterior
pituitory extract have also been reported   But it is to be noted that in
Ogden, Brown and Page's (15) rabbits, which developed hypertension slowly
and progressively after constricting the renal artery, an increased response
to pitressin (250 milli-units) preceded hypertension , and that Brown and
Maegraith (2) found that the increased responses to adrenalin and post-
pituitary extract developed by the 3rd, and to tyramine by the 4th day after
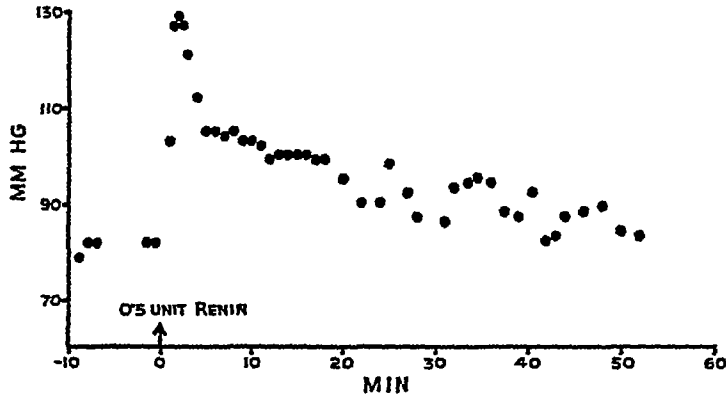renal artery constriction

Fig 4   Rabbit 7C   The response to 0 5 unit renin 46 hours after excising the sole and ischæmic
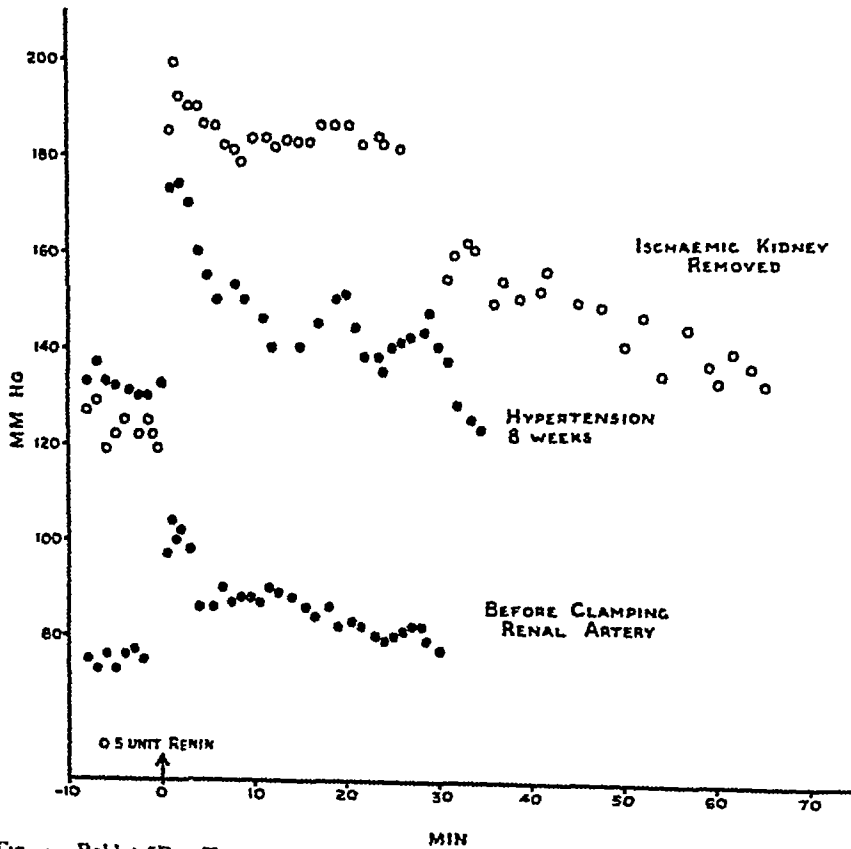left kidney   Duration of renal ischæmia and hypertension, 7 days

Fig 5   Rabbit 5B   Shows the responses to 0-5 units renin   (a) on 5 8 43 before constricting
left renal artery (black discs lower chart)   (b) on 6 10 43 2 months after constricting left
renal artery (black discs upper chart)   (c) on 9 10 43 48 hours after excising the left kidney
(open circles upper chart)   the right kidney had been removed 2 weeks before the left renal
artery was constricted

The effect of excising the sole ischæmic kidney   The response to renin after excising the sole ischæmic kidney depends on the duration of the preceding renal ischæmia and associated hypertension   When the hypertension has lasted 6 or 7 days, the response of the nephrectomised animal to renin (Fig 4) is similar in size and duration to that of a nephrectomised animal that has never had hypertension   Thus in the four normal animals of Table II nephrectomy increased the response to renin 1 5, 1 3, 1 4, and 1 7 times, while in the four animals of Table III with hypertension of 6 or 7 days duration the response after nephrectomy was 1 3, 1 7, 2 0, and 1 2 times as large as the response before renal artery constriction

When, however, hypertension has lasted 9 weeks or more, the response after nephrectomy is much greater and lasts longer (Fig 5)   Thus with one

TABLE IV

The effect of excising the ischæmic kidney in rabbits with hypertension of 9 weeks duration on the response to small doses of renin

Rabbit	Response in the ninth week before excising the ischæmic kidney				Responses after excising the ischæmic kidney				
	Dose renin	Initial B P	Rise B P	Dura tion	Hours after nephre ctomy	Dose renin	Initial B P	Rise B P	Dura tion
6B	units 0 05	mm Hg 102	mm Hg 22	min 11	9	units 0 05	mm Hg 88	mm Hg 48	min —
	0 05	109	32	16	31	0 05	93	56	55+
	0 05	120	6	3	—	—	—	—	—
8B	0 05	133	7	2 5	9	0 05	98	31	13+
	0 10	132	34	8	30	0 05	138	26	8+
5B	—	—	—	—	48	0 05	115	40	55

exception the responses after nephrectomy are greater in group 2 than in group 1 of Table III, and in the exceptional rabbit, 8B, the initial pressure was very high   In the two rabbits, 5B and 6B, where responses were obtained to the same dose of renin before renal ischæmia and after nephrectomy the responses were increased 2 9 and 1 9 times   In addition the response in 4B after nephrectomy was 5 4 times as large as in the first week after renal artery constriction   In three animals 4A, 5A and 4B, the response was much longer than we have seen in the rabbit in any other circumstances and our experience covers several hundred responses to doses of this order

Table IV shows the responses to small doses of renin before and after removing the ischæmic kidney at the 9th week   The responses to such a dose after nephrectomy are of the same order as those to 10 times the dose in a normal rabbit   A comparison with the response of the same animal before

the renal artery constriction was not made, but in two normal animals of the same litter as 5B, 6B, and 8B, and in the same week as their responses after nephrectomy were obtained, 0 05 units renin gave rises of 6 and 11 mm Hg lasting 5 and 6 min. respectively These results again emphasise the exceptional sensitivity to renin of the nephrectomised chronic hypertensive rabbit, and it is possible that this may prove a useful preparation in the assay of minute quantities of renin

Comment The original point of these experiments was to decide whether the persistence of the hypertension after excising the ischæmic kidney was due to a loss of the ability to inactivate renin It is clear that this is not the case, for despite the large and long responses to injected renin, the arterial pressure has in time returned to the pre-injection level in every case Yet the hypertension persists The persistence of the hypertension despite the absence of the kidney could only be explained on the renin hypothesis by supposing that renin were continuously released from a source other than the kidney For this there is no experimental sanction, for extracts made from organs other than the kidney of chronically hypertensive rabbits have failed to reveal the presence of renin (20)

These experiments have however revealed a further point of difference between the behaviour of rabbits with hypertension of short and long duration, namely the greatly increased sensitivity to renin shown by the animal with prolonged hypertension after excision of the sole ischæmic kidney As has been seen, in hypertension of short duration, the response to renin after nephrectomy is similar to that of a nephrectomised animal which has never had hypertension, while in hypertension of long duration, the response after nephrectomy is considerably increased and prolonged. While it is possible that this increase in response is the sum of the effects of prolonged hypertension and of nephrectomy on the reactivity to renin, the change in response indicates once more that the behaviour of the cardiovascular system in chronic hypertension has been modified by factors which are outside the kidney Three kinds of change affecting the cardiovascular system may arise during the course of prolonged hypertension namely structural changes in the heart and vessels, changes in cardiovascular reflexes or changes in chemical constitution Of these possibilities only the first has been examined

Structural changes in the heart and vessels

It has long been supposed, although on imperfect evidence, that in man hypertension at first remediable may become permanent through the development of structural changes in the vessels The similarity between this concept and the effect of time on the response of the rabbit to excising the ischæmic kidney has led us to examine the vessels for structural changes

in 4 rabbits of group 1 and 6 rabbits of group II Pieces of heart, liver and spleen, and in many animals stomach, small and large gut and adrenals were fixed in formol saline and sections stained with hæmatoxylin and eosin, hæmatoxylin and Van Giesen's stain, and Weigerts stain for elastin Not more than 3 sections of any tissue were examined and the arteries ranged in size from the smallest vessels with a recognisable muscular wall to the branches of the coronary arteries and the branches entering the mesenteric border of the gut Intimal lesions were not found in any sections Acute necrosis of the media was found only in one small branch of the hepatic artery in 5B, and in a small artery entering the mesenteric border of the small intestine in 8B These two rabbits were amongst the three with arterial pressure of over 130 mm Hg before nephrectomy , in the third, sections of heart, liver, spleen and kidney only were examined This is in agreement with a previous finding (27) that arteriolar necrosis only occurs in rabbits with a greatly raised arterial pressure The absence of intimal thickening in all, and of qualitative medial change in most, arteries examined in the animals of group 2 suggests that an irreversible vascular change is not responsible for maintaining the arterial pressure after excising the ischæmic kidney

Medial hypertrophy of the arteries has been found in human hypertension and has been detected in dogs with hypertension following renal artery constriction (3, 7) Many of the tissues examined in this series have been unsuitable for deciding whether or not medial hypertrophy has occurred Thus inflammatory changes and extensive hæmorrhage, presumably of uræmic origin were found in the gut of many animals , the widely dilated vessels in such tissues could not be compared with the more constricted vessels seen in other sections

Cardiac hypertrophy has previously been shown to occur in rabbits with persistent hypertension following renal artery constriction (19) In the present series the relationship between heart weight and carcase weight was not comparable to the ratio in normal animals, because after total nephrectomy the animals ate and drank little, though sufficient was offered Nevertheless, the animals with short and long continued hypertension, described in this paper, are themselves comparable in this respect, and the relationship of heart weight to carcase weight, in those animals where these weights were obtained, is shown in Fig 6 It may be seen that as in normal animals, heart weights are relatively greater in animals with low carcase weights, but that for a given carcase weight the heart weight is greater in those animals with long than in those with short hypertensions This difference may be correlated with the greater arterial pressures obtained in animals with hypertension of some weeks duration While it seems reasonable to suppose that cardiac hypertrophy is a process which requires time, we are unable for the reasons given to say from these figures whether any enlargement of the heart had occurred in those animals with hypertension lasting no more than a week

From these results it is concluded that qualitative changes in the vessels, such as arteriolar necrosis, which lead to narrowing of the lumen, are not the cause of the phenomena described in this paper   Nor can cardiac hypertrophy be held responsible for all we have observed.   For while it is reasonable to expect that the magnitude of the rise of arterial pressure due to a pressor substance such as renin may be related to the power of the heart, cardiac hypertrophy can not account for the persistence of the hypertension after excising the ischæmic kidney nor for the long duration of the pressor response to renin after nephrectomy in animals with chronic hypertension
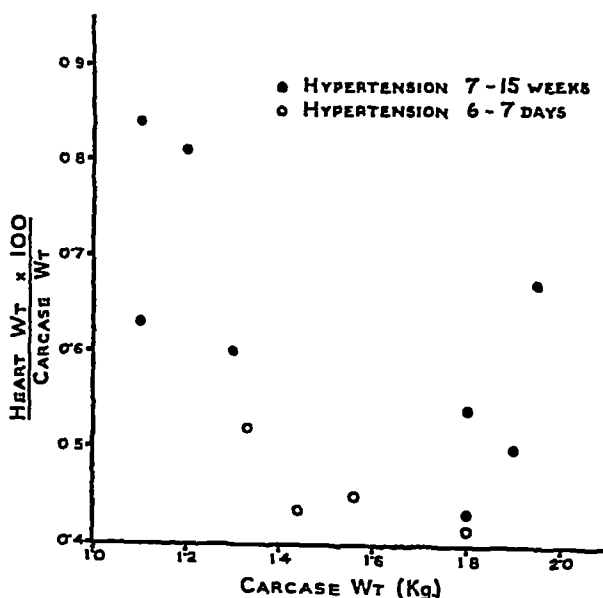
Fig 6   Shows the relationship of $\dfrac{\text{Heart wt} \times 100}{\text{Carcase wt}}$ to Carcase wt in 8 rabbits with hyper
ten ion of 7 to 15 weeks duration (black discs) and in 4 with hypertension of 6 to 7 days duration open circles   All animals dying 49 to 76 hours after total nephrectomy

DISCUSSION

The results recorded here augment the already considerable body of evidence suggesting that in the rabbit at least, the mechanism of hypertension following renal artery constriction is by no means as simple as at one time seemed probable   The suggestion is that while at first a renal humoral mechanism is solely or chiefly responsible for the raised pressure, as time goes on some factor or factors independent of the kidney play an increasing role in maintaining the raised pressure   The evidence for this suggestion may now be summarised

In the first week after constricting the renal artery, it has been here shown that the hypertension is usually abolished completely by removing the ischæmic kidney The time taken for the arterial pressure to fall to its normal level is of the same order when the ischæmic kidney is removed as it is after stopping an intravenous infusion of renin lasting 4 hours (11) Finally the renin content of the ischæmic kidney is at this stage increased (20) * These facts indicate that the kidney is solely responsible for the hypertension and suggest that the release of renin from the ischæmic kidney is the mechanism involved

Seven weeks or more after constricting the renal artery excising the ischæmic kidney does not abolish hypertension The maintenance of the hypertension is not due to the activity of the other kidney, since this had been removed previously in these experiments Nor is it due to failure to inactivate renin, nor apparently to production or storage of renin elsewhere, since no evidence of this has been obtained (20) It seems that a factor quite outside the kidney is responsible for maintaining the hypertension after nephrectomy, and it is not too much to assume that this same factor must have had an important, if not the chief, part to play in maintaining the hypertension while the ischæmic kidney was yet in the animal's body Although this new factor is not renal, it is clear that its development must ultimately be traced to constricting the renal artery To this new non-renal factor or factors we also attribute the excessively large and prolonged responses to renin observed after excising the ischæmic kidney in the rabbit with chronic hypertension and possibly the gradual and progressive rise of arterial pressure after clamping the renal artery, to which we have previously drawn attention (19) This conception of a non-renal factor playing an important role in chronic hypertension is in harmony with our previous observation of a normal renin content of the ischæmic kidney in rabbits with hypertension lasting 2 months or more (20) It is also in harmony with the evidence which Taggart and Drury (23) brought forward against the renin hypothesis of hypertension, for Professor Drury has informed me that the animals used in these experiments had had hypertension lasting some weeks Taggart and Drury's chief objections to the renin hypothesis were that in their rabbits with hypertension, the response to injected renin was not decreased, as it was in animals whose pressure had been raised by infusing renin, nor was the hypertension reduced when the responsiveness to renin had been abolished by injecting massive doses of the substance

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* In our paper describing the amount of renin in the kidney, as determined by biological assay, the conclusion was reached by inspecting the figures that constriction of the renal artery with consequent hypertension raised the renin content of the kidney in the first week I am indebted to Mi Wooton for a statistical investigation of this conclusion He informs me that our results show (1) that there is a significant rise in the renin content of the left kidney as compared with the right in the control experiments summarised in Table II (2) that the difference between the renin contents of the left (ischæmic) and right (normal) kidneys in animals with hypertension of 2 8 days (Table III) is significantly greater than in the control experiments of Table II (S = 0 50 , t = 5 0 , P = 01) Thus the conclusion stands the test of statistical treatment That the renin content in chronic hypertension is normal, is so clear from our figures as to need no mathematical analysis

The hypothesis here brought forward is based on the behaviour of the rabbit alone Superficially the position in the rat may seem rather similar This animal is peculiar in that constriction of one renal artery may produce gross and persistent hypertension even although the other kidney has not been touched In 1941, Wilson and Byrom (26) showed that in about two thirds of their animals, hypertension persisted after excising the ischaemic kidney They attributed the persistence of the hypertension to the activity of the other kidney whose blood flow had been reduced by arteriolar lesions, for they found that the extent of these lesions and of the glomerular damage was approximately proportional to the degree of residual hypertension At about the same time Friedman, Jarman and Klemperer (5) reported that hypertension produced by wrapping one kidney in cellophane frequently persisted after removing that kidney, and attributed the residual hypertension to irreversible changes such as in the vessels , in their animals the degree of residual hypertension was related to the intensity but not to the duration of hypertension before nephrectomy In 1942 Patton, Page, and Ogden (16), producing hypertension by constricting one renal artery, the other kidney remaining intact, found that the frequency with which excising the ischaemic kidney abolished the hypertension was not related to the degree but depended on the duration of the hypertension, occurring in 10 out of 21 animals with hypertension lasting 5-10 weeks , they, too, attributed the residual hypertension to changes in the unclamped kidney More recently Reed, Sapirstein, Southard and Ogden (19) have found that hypertension produced in the rat by Wilson and Byrom's method is, if recent, uninfluenced by nembutal anaesthesia or yohimbine hydrochloride, but, if of long standing, is reduced or abolished by these procedures , they infer that in the rat hypertension of long standing, but not of recent origin, is due to a vasomotor nervous mechanism The only experiments at all resembling those described in this paper are those of Grollman, Harrison and Williams (10) in which it is reported that hypertension produced by partial removal of, or application of silk around, the sole remaining kidney is not abolished during the second and third day after excising the affected kidney , in these observations the duration of hypertension is not stated but apparently exceeded 10 days It is still uncertain therefore whether the rat develops a non-renal factor in hypertension as does the rabbit

Despite very extensive experiments there is no suggestion of the ultimate intervention of a non-renal factor in the dog Several observers have reported that when hypertension is produced by constricting the renal artery to one kidney (1, 6, 25), the other being intact, excision of the ischaemic kidney restores the arterial pressure within some hours, even after a hypertension of 9 *months* as in one of *Goldblatt's* dogs (6) Rodbard and Katz (22) showed that when a normal kidney was left in situ, excision of the ischaemic kidney restored the arterial pressure to normal in 6, 6, 4, 4½, 3½ and 4½ hours in animals whose renal artery had been clamped 16, 5, 28, 10, 10, and 18 days previously When the sole and ischaemic kidney, or both kidneys,

were removed, the arterial pressure returned to normal in 50+, 10½+, between 12½ and 21, 9, 13, 40, and 15 hours in animals whose renal arteries had been clamped respectively 116, 27, 11, 24, 2, 13, and 2 days previously These figures reveal no clear correlation between the duration of renal ischaemia and the duration of hypertension following excision of the kidney

The relevance of the conception developed in this paper to the problem of hypertension in man is not yet apparent But it is worthy of note that in man a well-defined difference between the state of the vessels of the hand in the hypertension of acute and chronic nephritis has been identified, and has led to the supposition that the mechanism of hypertension in these two stages of this disease is not identical (17)

We are as yet in the dark as to the nature of the non-renal factor or factors which seem to play so important a part in the maintenance of hypertension some weeks after renal artery constriction in the rabbit Structural vascular changes, other than medial hypertrophy, are not necessarily present, though when present they may obviously play a part, and cardiac hypertrophy is not an adequate explanation The factor or factors obviously require time for their development and changes in the nervous or chemical mechanisms regulating arterial pressure are possible Even in the normal animal these mechanisms are yet imperfectly understood, as in the maintenance of the arterial pressure after total sympathectomy

SUMMARY

1   When hypertension is produced in the rabbit by constricting one renal artery, the other kidney having been removed, excising the ischaemic kidney after 8 days or less usually abolishes the hypertension in a few hours, excising the ischaemic kidney after 7 weeks or more has no effect on the hypertension during the period, not exceeding 4 days, the animal survives

2   The persistence of the hypertension after excising the ischaemic kidney in animals with prolonged renal ischaemia is not due to an inability to inactivate renin

3   The response to renin is increased in size and duration in normal animals by total nephrectomy Animals with renal ischaemic of short duration respond to renin after nephrectomy in the same manner as nephrectomised animals which have never had hypertension In animals with prolonged renal ischaemia the response to renin after nephrectomy is unusually great and prolonged

4   It is concluded that in the rabbit during the first week following renal artery constriction hypertension is due solely or chiefly to the release of a humoral agent, probably renin, from the ischaemic kidney Later a new and non renal factor plays an important, and perhaps the chief, role in maintaining the raised pressure

5   Qualitative structural changes in the vessels, such as arteriolar necrosis, may be absent in animals with prolonged hypertension in which excising the ischæmic kidney fails to abolish hypertension

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HEART FAILURE AND BONE BLOOD FLOW IN OSTEITIS DEFORMANS

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WHEN Sir James Paget (12) described the disease of bone associated with his name, he thought the bones were hyperæmic and, inclined to favour an inflammatory cause, he used the term " osteitis deformans " From the pathological point of view Cone (4) also stressed the highly vascular state of the affected bones, and noted the frequent association of chronic cardiovascular disease , he thought the bone pathology might be secondary to local vascular changes   It is well known that the skin over an affected bone is warmer than normal, the differences of temperature having been recorded by Klippel and Weil (9) and by Snapper (15)

The frequency of cardiovascular complications has been observed by various authors   One of Paget's original cases was noted to have a dilated heart at post mortem, and this was ascribed to calcification in the mitral valve   Hurwitz (7), recording 6 cases, noted that 2 of these showed evidence of heart failure   Kay, Simpson and Riddoch (8) were struck by the frequency of high pulse pressures (*see* Table I)   Out of 33 cases 14 had pulse pressures exceeding 60 mm Hg , in 7 the pulsations were heard down to zero pressure, an aortic diastolic murmur being heard only in 2   Cardiac enlargement was often noted, as were systolic murmurs over the præcordium   Seventeen cases seen at this hospital in recent years have been analysed and show similar results   cardiac enlargement in 6 and high pulse pressures in 6 The high pulse pressures are not accounted for by hypertension, as we have put cases with diastolic pressures over 90 mm Hg into a separate group Arteriosclerosis has often been invoked to explain such findings, and it is to this cause that Kay, Simpson and Riddoch, and Snapper ascribe the cardiovascular changes

TABLE I

Blood pressure and pulse pressure readings in cases of Paget's disease

	Kay, Simpson and Riddoch 33 cases	Present series 17 cases
Normal blood pressure	9	8
Hypertension, diastolic pressure over 90 mm Hg	10	3
Pulse pressure, 60 75 mm Hg	1	3
Pulse pressure over 75 mm Hg	13	3
	14	6
i e high pulse pressure in	40%	
Cardiac enlargement noted clinically	14	6

In this paper we report a study of congestive heart failure in a case of Paget's disease, together with evidence indicating a greatly increased blood flow through the affected bones

Case Report

A H , a man aged 66, was admitted to hospital on 22 9 44

History In 1930 a hard swelling appeared over the left ulna at the wrist In 1936 he noticed that his right leg was bowed outwards , 6 months later it began to be painful In February, 1941, he sustained a pathological fracture of the right humerus while shovelling, satisfactory union was obtained in about 12 weeks In February, 1943, left thigh deformity was noticed while in hospital On questioning he stated that his legs had swelled since 1941, he had been breathless on exertion for the last few years, with attacks of paroxysmal nocturnal dyspnoea for 3 months In February, 1943, he had been in this hospital with congestive cardiac failure of unexplained etiology

Examination showed a large skull, broad in frontal, temporal and parietal regions, marked thickening of right humerus, with limitation of right elbow movement after fracture, thickened left ulna, marked thickening and lateral bowing of both femora and forward bowing of the right tibia, the left leg being shorter than the right There was gross oedema of legs, thighs and sacrum In the cardiovascular system, there was thickening of the fundal arteries with some compression of the veins, and the radial arteries were slightly thickened There was a pulse of large amplitude and good volume, rate 75, rhythm regular The blood pressure was 140/70 The extremities were always very warm and capillary pulsation could be detected in the nail beds The jugular venous pressure (Lewis's method) was raised, the heights above the manubrium differing on the two sides, 1 2 cm right and 4 6 cm left, with free pulsations Cardiac enlargement was moderate, the cardiac impulse being 4¼" from the midline in the 5th space A systolic murmur was present over the whole præcordium The liver was slightly enlarged and tender, free fluid being present in the abdomen The chest was slightly kyphotic and basal crepitations were heard Examination of the central nervous system showed bilateral extensor responses with absent vibration sense in the left leg and impairment of pinprick appreciation up to level of T 5 6 Juxtapapillary choroiditis was present in both fundi Urine S G 1020 No albumin or casts were present

Investigations Plasma pho-phatase readings were 82, 116 and 96 (alkaline) and 7 2 (acid)
King Armstrong units   Serum calcium = 9 2 mg %   Serum phosphorus = 4 7 mg %   Plasma
proteins = 6 5 g % with 4 2 g albumin, 2 3 g globulin, and A/G ratio = 1 8   Blood count
Hb 83% (Haden)   R B C 4 100 000   W B C 4 000   Neutrophil polymorphs 60% lymphocytes
34%, monocytes 6%   Wassermann reaction was negative   Chest X ray showed heart transverse
diameter = 6¼ with left ventricular enlargement and prominent aortic knuckle   An electro
cardiogram was within normal limits   Skeletal X rays showed Paget s disease of bone involving
skull, lumbo-sacral spine pelvis both humeri left radius and ulna, right scapula and clavicle
right 2nd metacarpal and os magnum both femora right tibia and fibula, right navicular right
terminal phalanx of the great toe, and left cuboid

The blood volume by Gregerson's method (6) was 7 5 litres

On 3 10 44 an attempt was made to study the case by the method of
right auricular catheterisation   Although the catheter was arrested at the
junction of the subclavian and internal jugular veins, cardiac pulsations were
exceptionally distinct on the manometer   Samples withdrawn from this
situation had a bright red colour approaching that of arterial blood and
showed high oxygen saturation on analysis   This in the presence of a
normal oxygen consumption suggested that the cardiac output was probably
very high   Intravenous catheterisation was repeated on 31 10 44   The
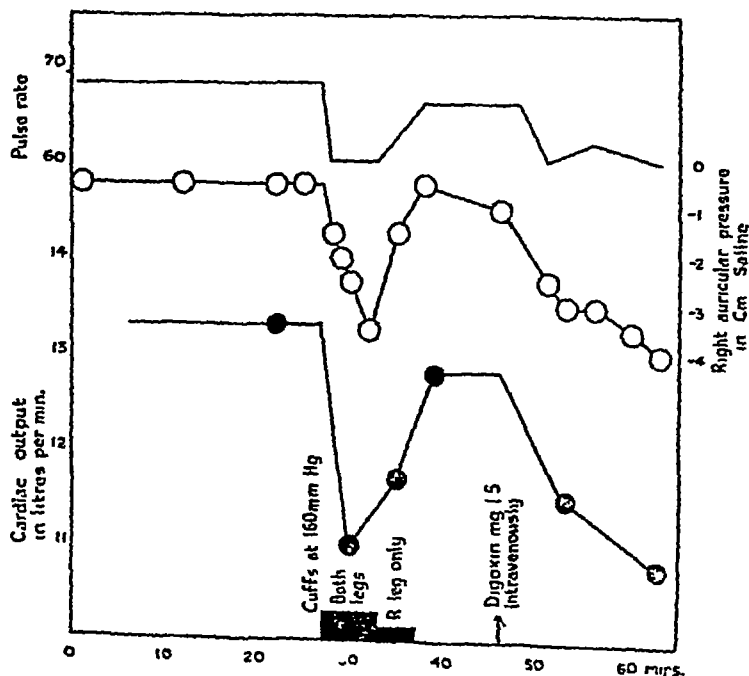
Fig 1   Shows the pulse rate right auricular pressure and cardiac output and their changes
in response to occlusion of the circulation through both legs by cuffs round the thighs
and to intravenous digoxin (Table II)

patient was propped up in bed at an angle of 45°, easy passage into the right auricle was achieved Right auricular pressure was raised (—0 5 cm relative to the sternal angle as compared with a normal —7 cm in the same position) The cardiac output was 13 3 litres/min In summary, then, we were confronted with a case of Paget's disease presenting the signs of cardiac failure, a pulse of high pressure and large amplitude, venous congestion and high cardiac output These cardiovascular phenomena are all features of cardiac failure in severe anaemia (13) and arteriovenous aneurysm The haemoglobin reading of 83 per cent at the time of the observation excludes the former cause, and no traumatic arteriovenous aneurysm was present It was thought, however, that free arteriovenous communications might be present, the blood being shunted through the affected bones

Certain measurable cardiovascular responses are obtained in patients with traumatic arteriovenous aneurysms Closure of the aneurysm leads to slowing of the pulse and a rise of diastolic blood pressure In generalised Paget's disease, closure of shunts in the bones is obviously impossible, but we tried the effect of complete occlusion of the circulation through the legs,

TABLE II

Data from which Fig 1 is constructed to show method of calculating cardiac output

Time	R.A P cm water	Pulse per min	B P mm Hg	A V oxygen* difference c c /litre	Oxygen consump tion c c /min	C O litres/ min	Remarks
22 min	—0 5	68	120/70	24 1	320	13 3	
27 „							Cuffs on thighs inflated to 160 mm Hg
28 „	—1 5	60					
30 „	—2 5	60	120/72	26 5	291	11 0	
33 „	—3 5	60					Left leg cuff released
35 „	—1 5		120/75	26 1	305†	11 7	
37 „							Right leg cuff released
39 „	—0 5	66		25 5	325	12 8	
46 „	—1 0	66					Digoxin 1 5 mg given intravenously
51 „	—2 5	60					
53 „	—3 0			27 0	310	11 5	
63 ,	—4 0	60		28 7		10 8	

* Arterial blood oxygen unsaturation 10 5 c c /litre Single sample taken and assumed to be constant throughout

† This oxygen consumption assumed to be midway between the two preceding values All other oxygen consumptions were measured on spirometer tracings taken at the particular times

by applying thigh cuffs at a pressure above systolic   The response obtained
is indicated in Fig 1, the mode of calculation being shown in Table II
The right auricular pressure fell with a slowing of the pulse and a fall in
cardiac output, the diastolic pressure rose very slightly   On another
occasion occlusion of the same limbs caused a rise in blood pressure
from 140/75 to 160/90, but the pulse remained unchanged at 56 per minute
These reactions, though variable, are somewhat similar to those we have
observed with partial occlusion of traumatic arteriovenous aneurysms
(unpublished data)

The effect of digoxin is also indicated in Fig 1   It lowered the venous
pressure and cardiac output to an extent similar to that produced by the

A = Plethysmograph

B = Arterial Occlusion

C = Venous Occlusion
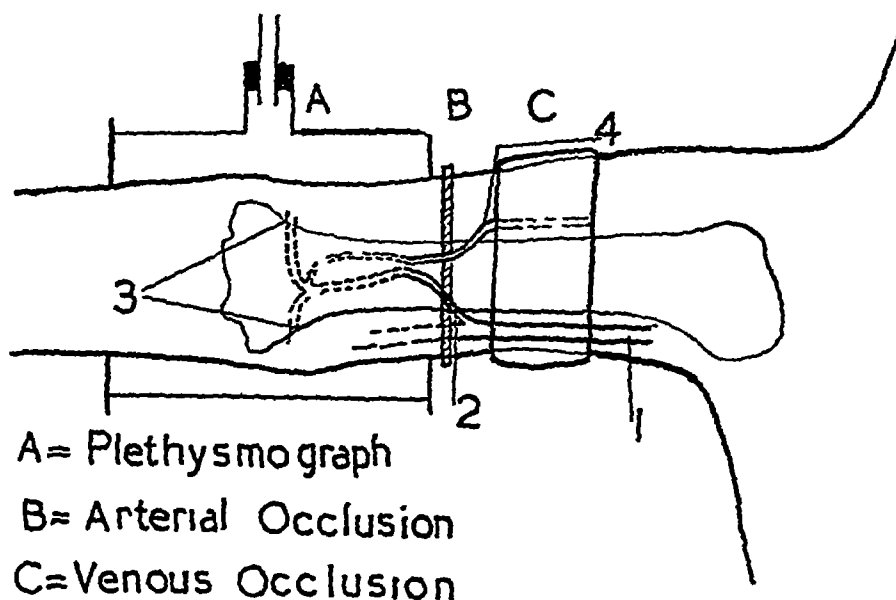
Fig 2   Diagrammatic representation of method for measuring bone blood flow   1 Brachial
artery   2 Nutrient artery entering shaft   3 Venous drainage from lower end of humerus
4 Venæ comites, running with nutrient artery shown as separate vessel for sake of clarity
When the arterial occlusion (B) is applied no arterial blood can flow past B except for the
blood flow in the nutrient artery as this vessel has already entered the bone and is protected
from pressure   Unless pressure is applied simultaneously in the collecting cuff (C) at
approximately diastolic level, blood can still drain from the bone through the venæ comites

preceding limb occlusion   McMichael and Sharpey-Schafer (11) have
shown that in subjects with normal hearts and certain conditions such as
severe anæmia and emphysema, in which cardiac output is raised, digoxin
lowers the cardiac output, probably by lowering venous pressure

The blood flow in bone   In view of the probability of an increased bone
blood flow it was decided to try to measure it

Method   Blood flow through a segment of a limb was measured with a Lewis-Grant plethysmograph, 6 inches long, with modifications as described by Barcroft and Edholm (3)   The plethysmograph and the limb were kept in air, precautions being taken to prevent cooling of the limb (4)   To measure bone blood flow the following procedure was adopted (Fig 2)   The plethysmograph (A) was placed over the lower end of the upper arm, enclosing the lower third of the humerus, the elbow joint and a small portion of the upper end of the forearm   An occluding cuff (not shown in diagram) was put round the arm below the plethysmograph, and the collecting cuff (C) was placed above, leaving a gap of approximately two inches between the lower border of the cuff and the upper plate of the plethysmograph   Just before applying the pressure to the collecting cuff the main arteries were occluded by tightening a rubber tourniquet (B) between the cuff and the plethysmograph
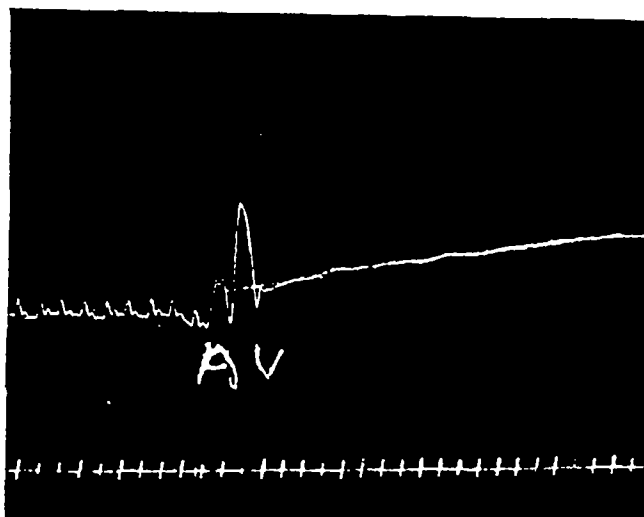
Fig 3   Bone blood flow in normal humerus
A and V mark the points of application of arterial and collecting pressures   These produce some transient distortion of the tracing, but thereafter there is a slow but steady rise, indicating the collection of blood in the limb distal to the arterial occlusion   The line drawn through tracing indicates the slope of the curve   In this, and subsequent tracings, the time record is in seconds

The blood supply of the lower end of the humerus is carried by two nutrient arteries, which are fairly constant in position, entering the bone just below the middle of the shaft   There are also numerous small vessels entering the bone through the periosteum, and the epiphysis has a few small nutrient arteries derived from the plexus round the joint   In normal bone the blood supply of the shaft is mostly obtained from the main nutrient arteries (1, 5, 10)   The venous drainage is through a number of small veins leaving the bone in the region of the epiphysis, and also by venæ comites running with the nutrient arteries   By applying bands as indicated in

Fig 2, blood collected in the soft tissues under the plethysmograph will represent not only that normally issuing from the epiphyseal veins, but also that diverted through these channels when the venæ comites of the nutrient arteries are obstructed by cuff C

Results

1 *Blood flow in normal bone* When the arterial occlusion (B in diagram) is applied, no measurable increase in volume is recorded in the plethysmograph When arterial occlusion is combined with a diastolic
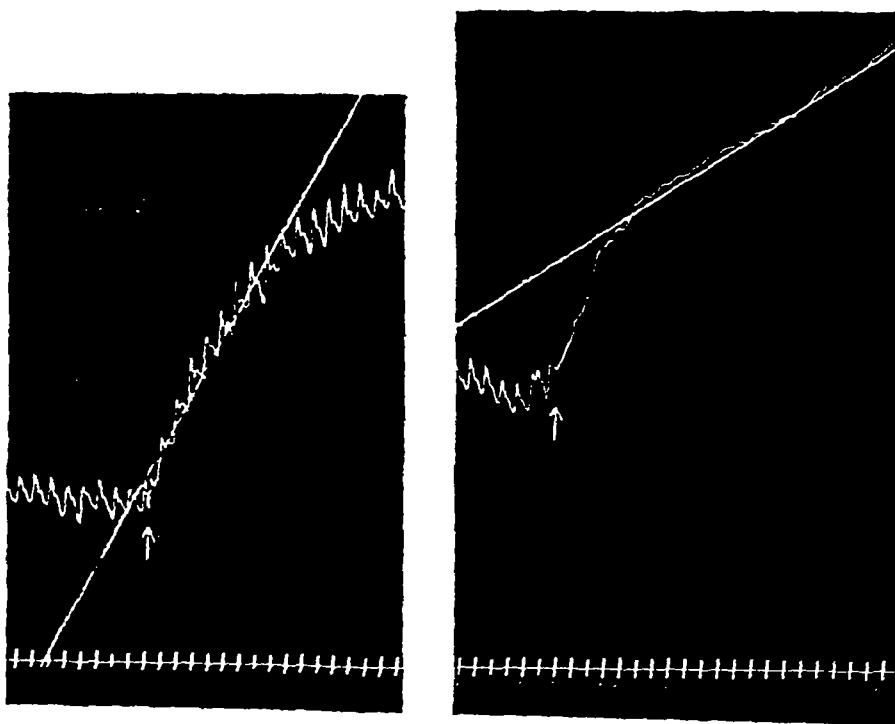

Fig 4   Bone blood flow in diseased humerus (osteitis deformans)   Subject A.H.
(a) Blood flow in elbow segment of arm.   Arrow indicated application of collecting pressure
(b) At arrow arterial occlusion + collecting pressure applied to arm.   Initial distortions due to application of pressure followed by a steady rise, due to bone blood flow   The slope of the lines drawn through the straight portion of the tracing indicates the rate of blood flow

pressure in the collecting cuff (C), a small increase in volume follows   On the other hand, applying an arterial pressure high up on the arm, *i e*, well above the point of entry of the nutrient arteries, does not produce any increase in the volume in the plethysmograph, whether the venous occlusion is applied or not (compare Fig 5)   These results seem to confirm the validity of the method   The volume of the humerus enclosed in the plethysmograph

was calculated from X-ray photographs taken in two planes   In normal
bone the total flow measured in this way varied from 0 5 to 1 0 c c /100 c c
bone/minute (Fig 3)

2   *Bone flow in Paget's disease*   The arm blood flow was measured in
the patient already described   The flow to the whole segment enclosed in the
plethysmograph was 2½-3 times the normal average   Measurement of the
bone flow in the diseased and enlarged humerus showed a very conspicuous
increase over the normal figures (Fig 4), averaging 10 c c /100 c c bone/
minute, a figure which represents the flow through the nutrient arteries
alone   The volume of bone was again measured by X-rays and the validity
of the method checked as already described (Fig 5)   In the same subject,
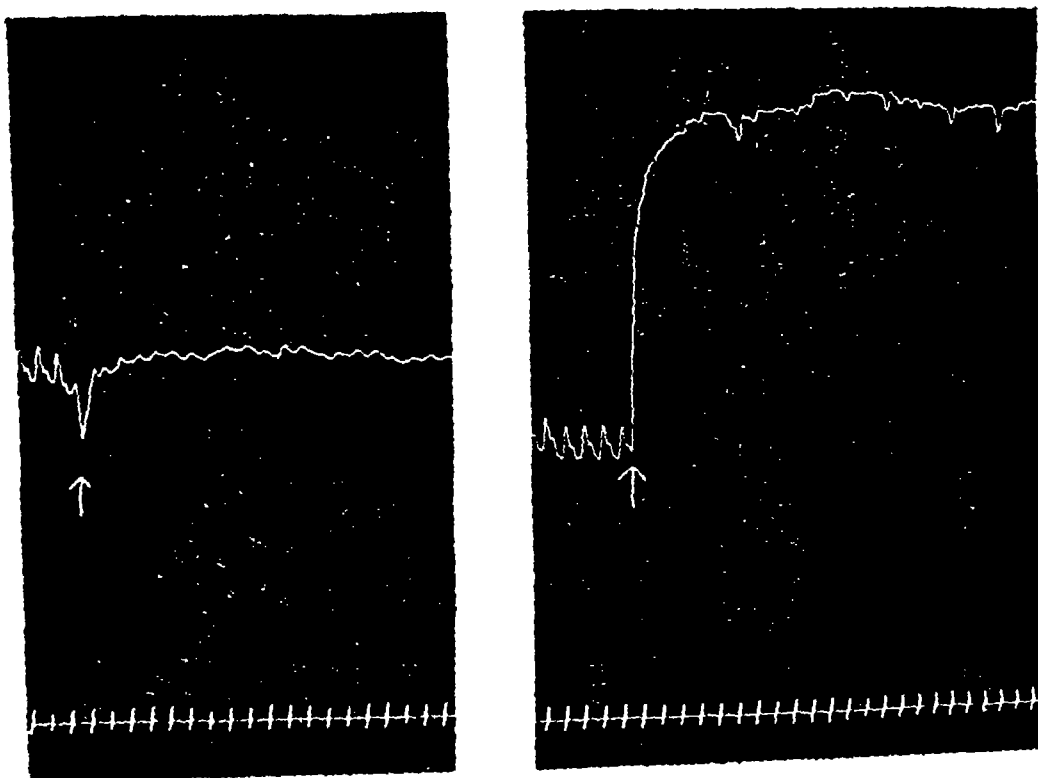
Fig 5   Subject A H
    (a) At arrow, arterial occlusion applied high up on arm   No measurable flow
    (b) At arrow, arterial occlusion, without collecting pressure, applied above plethysmograph
        No flow recordable

a comparison was made of the flow in the right and left legs   On the left
side the tibia and fibula showed no abnormality, on the right side both
were grossly affected   The total blood flow was increased on both sides,
but on the right side the blood flow was 8 8 c.c /100 c c leg/minute, whereas
on the left side with normal bones, the flow averaged 3 9 c c /100 c c leg/
minute   (Normal average 2 c c /100 c c leg/minute   *see* Cases 2 and 3
below)   No direct measurement of the bone flow was made in the legs, as
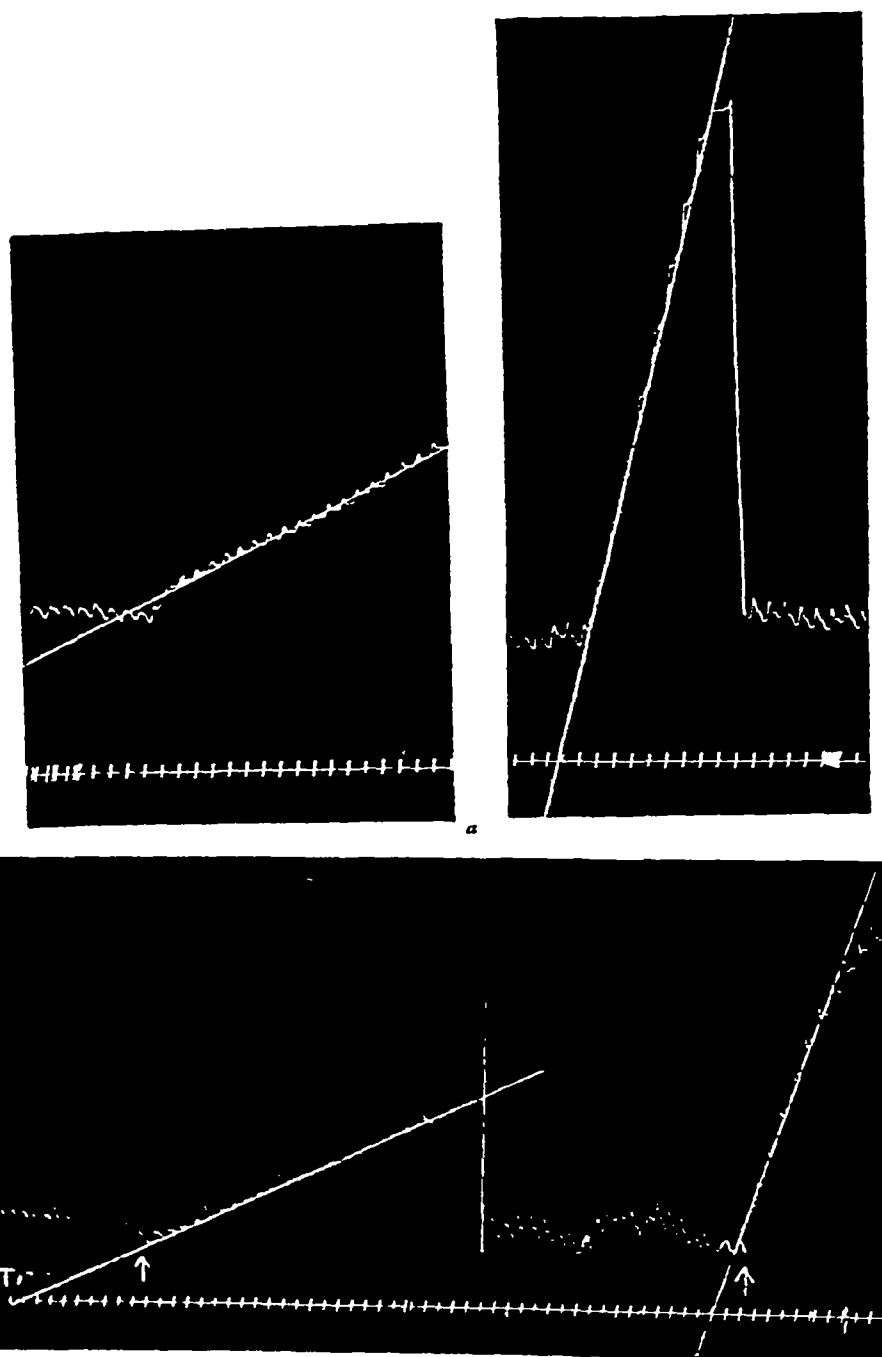
Fig. 6. Comparison of flow in normal and diseased legs in two subjects with unilateral osteitis deformans of tibia.
    (a) i Blood flow in normal leg ii Blood flow in contralateral diseased leg
    (b) i Blood flow in normal leg ii Blood flow in contralateral diseased leg
Note the great increase in flow in both cases in the diseased limb as indicated by the steepness of the lines drawn through the tracings

it is not possible effectively to occlude the main arteries which, running between the tibia and fibula, are protected from external pressure Let us assume that the difference in flow on the two sides is due to the increased -bone blood flow   The total flow through the segment of leg enclosed in the plethysmograph was 51 5 c c /minute in the normal limb and 125 c c /minute in the diseased limb   The bone volume in the diseased limb was estimated to be 370 c c , so the blood flow through this bone was 125-51 5 c c /minute, or approximately 20 0 c c /100 c c bone/minute

Further evidence of increased bone blood flow in localised Paget's disease has been obtained in two cases with unilateral involvement of the tibia In both cases the cardiac output was within normal limits

Blood flow in c c per 100 c c leg per minute

	(a) normal leg	(b) diseased leg
Case 2	2 2	10 (Fig 6a)
Case 3	1 7	11 5 (Fig 6b)

Applying calculations similar to that above, the bone flow in the diseased leg of Case 2 was 24 5 c c /100 c c bone/minute and in Case 3 was 26 5 c c /100 c c bone/minute   Tracings from these two cases are shown in Figs 6a and 6b

Quantitative considerations   These results establish clearly a very great increase in blood flow through bones affected by Paget's disease   The direct method employed measures the flow through the nutrient arteries only   In comparing the flow through a normal leg with the flow through the opposite leg affected by osteitis deformans, it was noted that the bone flow might be increased to 20 c c /100 c c bone/minute, as compared with a direct reading of 10 c c /100 c c bone/minute   This difference might be accounted for by the periosteal blood supply   Assuming similar proportions of nutrient artery and periosteal blood supplies in the normal limb, the measured flow of 0 5 c c /100 c c bone/minute may well be about half the normal total bone flow   We therefore estimate the blood flow through normal bone to be of the order of 1 c c /100 c c bone/minute, while that through Paget's bones is about 20 c c./100 c c /minute   Using these figures, the total blood flow to the skeleton has been calculated   The average proportion of the skeletal weight to that of the whole body is 15 % according to Skelton (14) or 17 6% according to Wilmer (17)   The average specific gravity of bone has been taken as 1 5 (16)   The body weight of the patient with generalised Paget's disease was 74 5 kg , so the normal volume for the skeleton in this case should be 7 45 litres   X-ray photographs of the whole skeleton were obtained, and an estimate was made of the increase in volume of the diseased bones -one by one   This was done either by comparing the photograph of the diseased bone with the comparable normal, for example, the enlarged right tibia with the normal left tibia, or, when this was not possible, a comparison was made with an X-ray of a normal bone from an individual of the same

height   The total skeletal volume in the case here reported was estimated to be 20 litres, of which only 3 4 litres were normal

In a normal subject, on these calculations, the total skeletal blood flow should be 74 5 c c /minute   This is probably an underestimate, as bones with an active marrow are more vascular than the humerus   In the patient with Paget's disease the 16 6 litres of diseased bone should receive a blood flow of approximately 3 3 litres, on the basis of a bone flow of 20 c c /100 c c bone/minute   If these figures are of the correct order, a considerable part of the augmented cardiac output is accounted for by the very large volume of blood flowing through the diseased bones   Although the rise in the cardiac output is not entirely due to the increased bone flow, it will be remembered that there was evidence of an abnormally high blood flow even in the leg with radiologically and clinically normal bones   An increase in the peripheral flow in tissues other than bone, together with the large skeletal blood flow would tally well with the measured rise in the cardiac output

Discussion

There has been remarkably little previous work on bone blood flow Drinker, Drinker and Lund (5) measured the blood flow through the tibia in dogs   They obtained high figures varying from 3 5 to 41 c c /100 g bone/minute   These figures are obviously inapplicable to man, as acceptance of the higher value would mean that more than half the total systemic flow would be through the skeleton

The data presented here indicate that there occurs in generalised Paget's disease an increase in bone blood flow of sufficient magnitude to produce effects on the general circulation similar to those resulting from free arteriovenous communications   Further work is required to study the relation between the increased flow and the radiological stage of the disease The absence of significant increases of cardiac output in cases of localised Paget's disease (Cases 2 and 3) suggests that it is only when the disease becomes generalised that the circulation as a whole is greatly increased, and in such cases the signs of heart failure may develop

Summary

1   A case of generalised Paget's disease presenting the signs of congestive heart failure is described   The cardiac output was 13 3 litres/minute There were phenomena comparable with *free arteriovenous communications* suggesting that this raised output might be due to increased vascularity in the affected bones

2   A method is described for measuring blood flow through the nutrient arteries of the humerus

3   The blood flow through the nutrient artery of the normal humerus is of the order of 0 5—1 0 c c /100 c c bone/minute   This value may only represent half the total flow, as the periosteal vascular supply is not included in the measurement

4   In Paget's disease, the bone blood flow is very greatly increased, up to 20 times the normal

5   In the case of generalised Paget's disease with heart failure the total skeletal flow was estimated to be 3 3 litres/minute

6   In two cases of localised Paget's disease there were no detectable changes in the general circulation, but blood flow through the affected limbs was greatly increased

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CONGENITAL TRICUSPID STENOSIS

By THOMAS LEWIS *

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THE case now described was under the care of Dr Vincent Bates of West Malling, Kent, whose clinical record follows, and who very kindly sent me the heart fixed in formalin

History of case

The mother of the child was a vigorously healthy woman of 31 years , she had already two fine children of 3¼ and 2 years, and had enjoyed perfect health throughout her last pregnancy There was no family history of heart affection, congenital or acquired

On February 3rd at an antenatal examination at the 32nd week, the normal foetal heart sounds were noted to be replaced by a loud swishing noise At subsequent antenatal examinations on the 6th, 15th and 22nd of the month, the same phenomenon was noted

On the 29th the mother began her labour, 3 days after the expected date Labour was natural, easy, and rapid, the 2nd stage lasting about 1 hour At birth the child, a girl, cried lustily and was vigorous, being of a normal rosy-red colour It weighed 8¼ lbs The cord was tied, and the baby put into the cot , 5 minutes later it was noticed to be very cyanosed Skin stimulation caused loud crying, but in spite of vigorous respiration, the cyanosis persisted On examination a systolic thrill was felt over the whole of the precordium, and a loud systolic murmur was heard with its point of maximum intensity one inch below and to the right of the left nipple The murmur was not heard in the neck, left axilla, or back of the chest By evening the baby's colour had improved considerably The hands and feet were warm and only slightly cyanosed

On the 30th, or day after birth, the baby was apparently making good progress Cyanosis was only slight, and breast feeding was satisfactory During the following two days there was little change, though feeds were not taken quite so readily The child's colour remained very slightly cyanosed On the 2nd of March the baby became very blue after crying, rapidly became worse despite remedies, was soon grey, restless, and semi-

conscious   The heart rate was regular at about 200 per minute   The colour did not improve, and the infant died four hours after the attack began

A half hour after death, a postmortem was done   On opening the pericardium, the sac contained about ¼ ounce of clear straw-coloured fluid The heart and lungs were removed intact and put into 5% formalin   The lungs were normal

The heart

The heart, when dissected clear, weighed 45 g   Both ventricles were enlarged, but especially the right, which was unusually dilated, and its apex came near to the apex of the heart   Both auricles were greatly dilated, but especially the right one   The ductus arteriosus was patent, passing a small probe

On opening the right auricle and right ventricle, their walls were found to be much thickened (Fig 1 and 2)   The opening of the inferior cava was higher than usual, its lower edge being 1½ cm above the A-V junction It led directly into a pocket formed by the fossa ovalis (Fig 2 *f o*) and what appeared to be the eustachian valve (*e v*), the latter greatly thickened and rounded at its free margin   Only at its upper end was this valve represented by a thin semilunar membrane   The rounded margin of the eustachian valve (*e v*) swept downward to become continuous with the margin of the septal segment of the tricuspid valve, which was similarly thickened and rounded throughout its length   The thebesian valve guarding the mouth of the coronary sinus was fenestrated (*c s*), its free margin twined around the margin of the eustachian valve   The foramen ovale was circular and 3 mm across, a valvular aperture at the front of the fossa ovalis   The septal segment of the tricuspid valve (*s s*) was closely bound down to the ventricular septum, short representatives of broad chordæ were attached to its thick rounded margin   The leaf of this segment was irregularly nodular

The remainder of the tricuspid valve lay free but was similarly and more grossly thickened, in this, chordæ and margin were not obvious, being replaced by what were seemingly warty thick excrescences covering the whole substance of the segment   The great thickening and nodular margin of the anterior cusp is well seen between the papillary muscles *P1* and *P2* of Fig 1, as is the nodular appearance of the main sheet of this cusp   The third cusp was represented by a mass of nodular tissue in which the papillary muscle *P3* ended   The aperture of the valve was oval and 12 × 9 mm in size, it was quite rigid and the stenosed valve must have been grossly incompetent

The conus was relatively small, and the entrance to it perhaps a little narrow   The three pulmonary valves were normal

The wall of the left was less thickened than that of the right ventricle The mitral and aortic cusps were normal, the chordæ of the former being

very numerous and even the finest of them (mere gossamer threads) intact The membranous septum was intact

The left auricle was much dilated but its wall of usual thickness and otherwise normal

Histology Sections were cut through the valve in a number of places at right angles to its free margin and stained with hæmatoxylin and eosin, and an elastic stain, the sections included the thick nodules on the cusp joining papillary muscles *P1* and *P2* of Fig 1, the septal cusp in the region of the letters *SS*, and the centre of the large papillary muscle including the warty looking thickened cusp to which this was closely joined above In none of these sections was there any appearance suggesting acute or chronic inflammation, or scarring Apart from muscular attachments and inclusions, all the remaining tissue was alike in being made up of irregular compact cushions of connective tissue in thick layers and masses, uniformly and rather richly studded with oval, elongated, or sometimes stellate nuclei, staining uniformly, the connective tissue fibres were arranged in parallel and massed to run in directions conforming to the contours and sometimes whorled All such masses of tissue were enclosed by a single layer of endothelium and sparse subendothelial layer, which showed the usual thin elastic laminæ, exactly similar tissue elements were found in sections of the normal mitral valve The cusps of the tricuspid valves and their nodules often proved less solid than had been thought In many places they were rendered sponge-like by the penetration of irregular and lengthy lacunæ, the spaces containing unchanged blood, being lined by endothelium, and communicating with the ventricular cavity These irregular spaces were sometimes crossed by primitive chordæ on the ventricular side, and these had the same histology as the surrounding masses of the main cushions Fig 3 which is representative of most of the valve tissue is of a section of the anterior cusp cut at right angles, it shows the uniformity of the connective tissue divided up by irregular lacunar spaces, all lined by endothelium Sections of the undeformed mitral valve presented connective tissue of precisely the same kind as that illustrated by this section The histological appearances are not those of a valve in which cusps and chordæ are matted together and thickened by the deposition of inflammatory tissue, but they are quite definitely those of an undeveloped or malformed valve It is as though the process by which the cushion has become moulded to form cusps and has become divided at its margin to form chordæ has been arrested, with the moulding incomplete and showing irregular and under-developed lacunæ between masses of connective tissue, thus left in the form of folds, irregular nodules, and primitive chordæ Fig 4 is of the central nodule lying on the edge of the cusp between *P1* and *P2* It shows the same type of primitive connective tissue, though the mass is more compact and the directions of the connective tissue fibres more variable This mass contained several wide vascular spaces and many narrow ones, sometimes very flattened out or branched, a number of the spaces were circular and of the width of capillaries All

without exception were lined by endothelium only   Again, there is here no evidence of inflammatory changes such as the nodular state of the cusp seemed to promise, but an appearance consistent with maldevelopment These two figures (Fig 3 and 4) sufficiently represent the histology of the tricuspid valve as a whole

The bases of the valve segments contained perfectly normal muscular tissue, as did the papillary muscles   There was nowhere any sign of necrosis, or calcification of the myocardium, whether in papillary muscle or in the rest of the heart

The ridge of tissue running up the septal wall of the auricle (e v Fig 2) proved to be in small part muscular, but consisted mainly and superficially of a long whorled bar of connective tissue, similar to that found in the thickened cusps, and again without any evidence of inflammation

Discussion

The specimen here described is important because it illustrates so clearly how a valve, congenitally malformed, may appear to be affected by an inflammatory lesion, resembling to the naked eye that following rheumatic endocarditis   It is concluded on two grounds that this particular lesion illustrates malformation and not foetal endocarditis   Firstly, the mass of the malformed cusps and chordæ when examined histologically consisted exclusively of primitive connective tissue, such as was to be found in the undeformed mitral valve in the same case , there being no signs of superficial inflammatory thickening, organised thrombus, or scarring   Secondly, the lesion of the tricuspid valve did not stand alone , above it in the right auricle was a curious deformity of the valves guarding the mouths of inferior vena cava and coronary sinus   This auricular deformity, illustrating faulty architecture rather than distortion of pre-existing normal structures, was continuous with the malformed septal segment of the tricuspid valve, and both presented a common histological pattern   Before proceeding to instance other cases of tricuspid valve defect, it will be convenient to examine briefly and as a more general question the role which endocarditis may be supposed to play in producing heart lesions of the new born

Foetal endocarditis   This has long been suspected to be the cause of certain cardiac defects, especially such as are not readily interpreted as instances of arrested development, but which may be supposed to result from processes implanted during the later months of pregnancy by which time the heart has normally assumed the fully developed form which it will retain till birth   Few would now conceive defects in interauricular or interventricular septum, or malposition of the main arterial trunks, as arising other than out of maldevelopment , naturally, it is in respect of a lesion found limited to, or concentrated upon, a valve, producing in it gross thickening and distortion, that endocarditis is especially prone to be regarded

as the causative agent   Lesions of this kind that are usually cited are
stenosis of the aortic, the pulmonary, the mitral, or more rarely the tricuspid
valve

The earlier material gathered to illustrate these cases and interpreted
as the result of fœtal endocarditis has now but minor value from this
standpoint, because interpretation was based upon what may be the fallacious
evidence of naked-eye appearances   As the present specimen clearly illus-
trates, and as many workers have previously emphasised, the origin of
cardiac defects found at or shortly after birth may be misjudged if the
minute anatomy remains unstudied   Fischer (6) was one of the first to insist
upon histological evidence, for nodular excrescences on the valves of the
newborn do not disclose the picture of an endocarditis, fresh or healed
Many others have followed suit in emphasising the need of microscopic
examination (Keith (11), Lewis and Grant (14), Ribbert (21)   Ribbert,
who believed fœtal valves to be relatively insensitive to infection, and who
was not prepared to acknowledge fœtal endocarditis other than as a very
rare malady, pointed out in 1924 that no instance of fresh verrucose or
malignant inflammation had been described , every care seemed to be due to
past processes despite fœtal endocarditis being assumed to occur in the
later and not in the earlier months of pregnancy   Fresh inflammatory
lesions would declare themselves at once   The outstanding fact about
these thickened, crumpled, nodular, but otherwise smooth valve segments,
is that sections show them to consist of just those cushions of primitive
connective tissue, which have been described in detail in our own case
Keith in 1909 expressed his view that pulmonary stenosis is not due to fœtal
endocarditis, precisely because of the primitive connective tissue shown by
sections of the body of the valves   The histological descriptions or figures
of Fischer, Lewis and Grant, Mönckeberg (17), Farber and Hubbard (4),
Stohr (23), and more recently Gross (9) and those now presented, all
substantially agree that the distorted valves are composed of this kind of
tissue   The essential normality of this tissue and the failure of any frank
display of inflammatory plastering together of normally formed cusps or
chordæ, or of infiltration, must weigh heavily, if not conclusively, against
the view that the lesions originate in fœtal endocarditis   Nevertheless,
so firmly has the latter idea become implanted that, failing direct evidence
of valve infection and inflammation, workers have not hesitated to call up
in its support the indirect evidence of lesions found in the ventricular
endocardium or muscle, omitting to note that such lesions—if indeed
inflammatory—would make the absence of clear signs of inflammation in
the valve itself all the more remarkable

To exemplify, Fischer (6), in his oft quoted case of aortic and mitral
stenosis, in a 5 week child, found in the valves themselves no evidence that
could be held to support deformity by inflammation, but unexpectedly
found a uniform richly cellular connective tissue , yet it was areas of necrosis,
diffuse fibrosis, and calcification of the ventricular myocardium, in the

region of the papillary muscles that he stressed in arguing for the inflammatory origin of all the lesions

A number of the reported cases of congenital aortic stenosis or atresia have exhibited in addition, conspicuous thickening of the lining of the left ventricle (3, 4, 8, 13, 18, 23 and 25)   It is the rule in such cases for the aorta above the stenosis to be hypoplastic, the ventricle may be hypoplastic too, and the ductus arteriosus open   These facts in themselves suggest an original fault of architecture, Willen and Beck's second case (25) can be regarded safely I think as subaortic stenosis   Whether all these hearts were malformed or not, the thickening of the endocardium, usually described as permeated by numerous wavy elastic fibres, seems to have run to type, though its explanation is disputed   For Willen and Beck it represents old parietal endocarditis, which, however, is unknown in the foetus and in the adult occurs only with a terminal infection   To Gross it is a simple hyperplasia, perhaps secondary to circulatory changes, perhaps developmental   One possibility, that I have not found named, is that the change, with underlying penetration of muscle by connective tissue strands, may be connected with deficient blood supply to the muscle of the left ventricle   The atresia of the aorta suggests this possibility, often blood could only reach the coronary arteries by passing back through the narrow aorta from an open ductus, the state of these coronary arteries is not usually mentioned, though Farber and Hubbard (4) say that they could find the left coronary artery of their first case in sections only   Whatever the cause to which thickened ventricular lining may be attributed ultimately, it is not known to be the result of inflammation and forms very indirect and unsubstantial evidence for inflammation of the valve

Another feature of these same reports (3, 18, 25) is the mention of unusual blood filled channels, either in the thickened valves, thickened ventricular endocardium, or deeper lying muscle, these are apt to be regarded as newly formed vessels, and are used to support the idea of inflammation   Actually it is unclear that they are blood vessels, it would seem much more probable that these vascular spaces are inclusions of the nature of sinusoids, for they are described as wide or even cavernous, and to be lined by a slender layer of endothelium   Such certainly were the spaces seen in our own specimen and illustrated by Fig 3 and 4

Tricuspid lesions   Lesions of the tricuspid valve, which the present case exemplifies, are, according to Herxheimer (10), among the least common

Peacock (19) described a livid child, presenting a precordial systolic murmur when seen at 3 months, the autopsy at 7 months showed a tricuspid valve thickened, adherent at the margins of the cusps, and consequently stenosed   Although the right ventricle was small, its septum was perforate, and the *pulmonary artery very large*   Kelly (12) described the heart of a 5 week child, who became cyanosed when crying   In this heart the tricuspid valve was very small and completely closed   Both interauricular and interventricular septa were perforate   Vernon (24) described the heart of a

child dying blue and breathless 4½ hr after birth   A festooned, imperfect diaphragm represented the tricuspid valve, which was judged incompetent The aorta arose unusually to the right over an imperfect septum   These three cases from the older records suffice to show that lesions of the tricuspid valve may be associated with anomalies now generally admitted to be defects of development

Cases of Finlay (5), Andrew (2), Raab (20), among the older series, exemplify associated defects of pulmonary and tricuspid   MacCullum (15) described at greater length the heart of a patient dying of tuberculosis at 30 years   The tricuspid valve was ballooned into the right ventricle, two of the cusps were wrinkled, folded membranes, fused to the ventricular wall and apparently functionless, the remaining cusp was membranous and formed a sort of intervalvular chamber, leading by a round hole into the large right ventricle   A peculiarity of this heart was the large right auricular appendix, opening by two mouths into the auricle   Ebstein's case is said to have been similar

In Griffiths' child (8) of 5 years, the tricuspid valve was profoundly malformed, the septal flap being quite isolated like an inverted cusp and without chordæ, the left flap is described as fixed at one commisure by confluent rudimentary chordæ, and for the rest obscured by adherency to large columns of ventricular muscle, the posterior flap had apparently no chordæ   This case is of exceptional interest in that there was a continuity of the eustachian and thebesian valves, bringing the mouth of the coronary sinus within that of the inferior cava   With our case it suggests that a single disturbance of development can break the continuity of the septal segment of the tricuspid valve, while simultaneously disturbing the normal relations of eustachian and thebesian valves

Malan's patient (16) presented an anomaly of the tricuspid valve, two cusps being much shrunken and the third sail-like without chordæ   It was possibly congenital in origin but its chief interest lies in the fact that the subject survived military service and land work till his 60th year

Sternberg's patient (22) died at 5½ months   The heart showed a stenosed tricuspid valve, the margins nodular   The pulmonary artery was closed, the right ventricle was minute   He accepted the soft plump excrescences as fœtal endocarditis without further examination   He explained the small right ventricle on the ground that it had lost its function   I have a specimen in my possession of complete pulmonary stenosis in a child dying at about the same age with tricuspid valve and ventricular septum intact, in which the right ventricle was large and very thickened with hypertrophy, it had equally lost its function   The small ventricle in Sternberg's case should be regarded as maldeveloped

Abbott's report (1) is of a cyanotic child dying at the 7th day, the septal cusp of the tricuspid was incorporated with underlying myocardium without chordæ, and carrying on its auricular surface a mass of irregular papillary endocardial outgrowths of gelatinous appearance, arranged in

rows and extending along the other cusps as well as on the free borders of the pulmonary segments   The structure of all these nodules was an abundant fibrillar matrix, covered by a single layer of endothelium, and showing no sign of inflammation or hyperplasia   This case report may be set against Sternberg's, emphasising as it does the occurrence of nodules in conditions of the endocardium that are non-inflammatory in origin, and thus impressing the insecurity of conclusions based upon mere inspection

The brief comments here attached to these case reports will suffice to bring the relation of foetal endocarditis to disease of the tricuspid valve into line with the more general discussion preceding it   The problem is in each case the same, and the evidence points similarly to the conclusion that foetal endocarditis as a causative agent has acquired too much prominence, the lesions found being predominantly those of faulty development

SUMMARY

An illustration of tricuspid stenosis in a newly born child is described, which, while resembling that resulting from endocarditis macroscopically, proved on minuter examination to be a congenital malformation   A brief review of previously reported cases of valves deformed in early life, including cases of tricuspid deformities, leads to the conclusion that these are usually, if not always, the result of congenital malformation

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Fig 1 Twice nat size Photograph of the heart with the anterior wall of the right ventricle
removed S V C = superior cava A = aorta Pu = pulmonary artery P1 P2 and
P3 = the three chief papillary muscles S.S = septal cusp of tricuspid valve

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In Memoriam

THOMAS LEWIS Kt, CBE, MD, DSc, LLD, FRCP, FRS,
Physician in charge of the Department of Clinical Research, University
College Hospital, Fellow of University College, London

Sir Thomas Lewis died in his 64th year at his home Clearburn,
Loudwater, near Rickmansworth, on March 17th, 1945 By his wish he was
buried in Llangasty Churchyard, Breconshire, overlooking Llangorse Lake,
where, from his boyhood, he had gone again and again to be with wild
creatures During his life he had accomplished much, he had won
international fame as a clinician and as a physiologist, his work had written
new chapters in the mechanism of the heart beat, in the behaviour of blood
vessels and in the mechanism of pain What perhaps gave him most satis-
faction at the end of his life was the knowledge that the cause to which he
had unsparingly devoted his life was won, that the ideal of clinical science
was inspiring men of ability, that opportunities for devoting a lifetime to
its pursuit had been created and were likely to increase, and that the men
whom he had inspired were united in a determination to further its end

Thomas Lewis was born in 1881 at Cardiff, the home of his
father, Henry Lewis, a distinguished mining engineer and colliery owner
As a boy he was educated largely at home, for his health had not allowed
him to remain at Clifton College to which he had gone at the age of eleven
He cared less for books than for roaming the countryside in search of living
creatures, and in particular birds It is perhaps to these early interests
that may be traced his unusual manual dexterity, and his immense capacity
for original observation, and to his early freedom from the conformity of
a large school may be attributed some at least of the independence of outlook
which was later to distinguish him so sharply from his fellows His medical
career began at University College, Cardiff, where, under the influence of
Swale Vincent, his first papers were written, amongst them in 1901 the
first of a series of papers on the hæmolymph glands which are still standard
descriptions For his clinical work he proceeded to University College
Hospital qualifying in 1904 and winning the University medal in the Final
M B examination and other prizes After being house physician to Thomas
Barlow and house surgeon to Victor Horsley, he was appointed to the staff
of the London Chest Hospital in 1907 But, although he had chosen a
career of consulting practice, the flame of scientific enquiry could not be
stifled, and he was soon at work in Starling's laboratory at University
College, applying the methods of physiology to an analysis of the phenomena
which he was encountering in his hospital patients Two papers in 1906 on

the influence of respiration on the arterial and venous pulse marked the turning point in Lewis's career, for in 1907 they brought him into contact with James Mackenzie, then newly arrived in London from Burnley

In later life Lewis spoke of no man with greater admiration and affection than of James Mackenzie, and it would seem that these feelings were returned by the older man, for when as a result of the activity of the group he had stimulated, the journal " Heart " was born, Mackenzie asked Lewis, then 27 years of age, to be its editor   The two had certainly much in common   Years later Lewis wrote " The life of Mackenzie was devoted with rare singleness of purpose to his adopted profession   The patient was the lodestone of all his work   Gifted with a vigorous and impressive personality, and possessed of unusual faculties of criticism, he was intolerant of authoritative statement and of traditional utterance, combative in argument, yet open to conviction   In him a mind turning incessantly from the known to the unknown, in him a vision ever searching the great veil   In discriminating known from unknown, his independent mind showed masterly power , in piercing the veil his vision was of unusual keenness   He possessed remarkably the gift of stimulating others , to know him intimately was to imbibe his spirit , his was a rare eagerness to impart knowledge "   These words might have been written of Lewis himself   There are remarkable similarities between the careers of these two great, perhaps the greatest, figures of British Medicine of the present century   Each left the rugged country of his birth and boyhood to do his life's work in England   Each had in full measure the ardent uncompromising spirit of the crusader, the covenanter, the reformer, and each sought the same end, the advancement of precise knowledge concerning disease and the abolition of the remnants 'of mediæval quackery   Even the subjects of their contributions to knowledge coincided, for in two of the three major fields of his work, the study of irregularities of the heart and of pain, Lewis followed Mackenzie   Finally, and by a strange irony, each fell a victim to the same disease, coronary artery thrombosis, to whose elucidation each had contributed so much   There were differences of course   Most of Mackenzie's work was done in the hours he snatched from a busy general practice , his work was of great and lasting importance to the outlook and to the practice of medicine, and particularly to diagnosis and prognosis   Lewis was associated from an early age with men of the calibre of Swale Vincent at Cardiff, Victor Horsley, Starling, Cushney and Bayliss at University College   He used more freely the methods of the physiological laboratory, and particularly the experimental method, on men as well as on animals, and his work is, therefore, the more detailed and searching and of greater consequence to the theoretical basis of medicine

Lewis's main scientific work may be divided into four periods   The first, directed to an analysis and understanding of irregularities of the heart, came from his meeting Mackenzie   The chief problem then engaging

Mackenzie's mind was to understand that total and persistent irregularity of the heart which he had shown to be peculiar in reacting to digitalis, which revealed no trace of auricular activity in polygraphic records and which he attributed to nodal rhythm   A new line of approach was needed and the string galvanometer recently introduced by Einthoven seemed promising   Lewis installed the new instrument at University College Hospital in an ill-ventilated cellar, meant for charwomen's implements, the only accommodation that could be provided for him   Results came soon As he himself relates, "in 1909 a man came to my outpatients suffering from paroxysmal tachycardia,   I won from him what, in that year, was unique, namely a complete series of venous and electrocardiographic curves in and out of the attacks   These records gave a startlingly plain record of true nodal rhythm "   " These records   were of regular tachycardia and thus sharply focussed attention on the weak point of Mackenzie's hypothesis namely its inability to explain the irregularities of the ventricle, they drew the focus of attention away from the intricacies of jugular curves where it had rested too long "   The first stage in the recognition of auricular fibrilla-tion in man came at the end of 1909 with a comparison of the electrocardio-graphic and polygraphic records obtained in human disease with those of the condition that had been experimentally produced by Cushney in the dog, revealing a long list of features identical in the two series, and providing a hypothesis explaining for the first time much previously obscure   It was characteristic of Lewis that he thought the evidence incomplete Learning that irregularity of the heart occurred spontaneously in old horses, he obtained records from animals tethered in the mews outside his laboratory and, finding them identical with those in man, followed an old horse to its slaughter on Salisbury plain where, when the chest was opened, he saw unmistakably displayed the auricle in fibrillation

Lewis now pressed forward with intense energy and success along this new path of research, so that all the world soon knew him as an investigator of the very first rank   Working sometimes alone, sometimes with collaborators visiting him for a year, he proved the origin and mapped the course of the excitation wave from its origin at the S A node to its final spread through the Purkinje tissue lining the ventricles, by timing the electrical changes in the several parts of the heart with astonishing precision In awarding the Copley Medal to Lewis in 1941, the President of the Royal Society (Sir Henry Dale) said of this work " Considered by itself this work ranks as one of the outstanding achievements of experimental physiology in our times, and it has given to physiology a large part of its present detailed knowledge of the nature of the heart beat "   It is all the more remarkable as being the work of a practising physician whose laboratory consisted of a single room   This work, published in 1914-15, was the subject of his Croonian Lecture before the Royal Society in 1917, and led to his election to the Fellowship in 1918   During this period his work laid down the

principles of electrocardiography, and of his conclusions only one, that of
the form of the human electrocardiogram in right and left bundle branch
block, has failed to stand the test of time, only one feature of major
importance has subsequently been added, that of the changing ventricular
complexes following myocardial infarction

The first World War broke in upon Lewis's electrocardiographic studies
and led to the second phase of his work In the winter of 1914-15
arrangements had almost been completed for him to command a Welsh
Military Hospital in Mesopotamia when he was invited by the Medical
Research Committee to take charge of work on "soldier's heart" This
work was carried out first at University College Hospital, to which he had
been appointed assistant Physician in 1912, later at Mount Vernon Hospital,
Hampstead, and finally at Colchester The work of Lewis and his team on
soldier's heart, or as it was re-named, effort syndrome, had several
important consequences It led to a clear description of the condition,
though not to its cause, and to its treatment by graduated exercise It
led to a scientific examination of the criteria for the diagnosis of
organic heart disease that was subsequently of great importance to
practice Further, finding himself deprived of his laboratory facilities,
Lewis began to employ the experimental method in observations on
the human subject, using the simplest apparatus, in a way that was
later to become so striking a feature of his work The most important
immediate result was his discovery of the independent contractility
of the capillaries published in 1917, a discovery contemporaneous with
that of Ebbecke And for himself it led to his entering the Medical
Research Council's full-time service in 1916, and thus abandoning finally
and without regrets all private consulting practice, on which, except for
the three years of his Beit Fellowship from 1910-12 he had previously
depended for a living For his work on "soldier's heart" and as
consultant to the Ministry of Pensions Lewis received the C B E in 1920,
and was knighted in 1921

This period, of the clinical study of heart disease and related conditions,
was completed after the war by the follow-up in his outpatient department
of 1,000 war pensioners with heart disease, concluded 10 years later by
Grant, with the loss of only 13 records, and with the follow-up of 500 cases of
effort syndrome for 5 years by the same worker, thus providing the most
complete records extant of the course of these diseases Lewis now returned
for a time to experimental electrocardiographic work, demonstrating that
flutter and fibrillation were the result of the excitation wave passing round
and round the mouths of the great veins, the so-called circus movement
described earlier by Mayer in the umbrella of the jelly-fish and by Mines in
preparations of cold-blooded hearts This brilliant scientific analysis
concluded, Lewis abandoned electrocardiography and never returned to it
He had, he felt, exploited to the full the new method, as he said "the cream

was off," and he had become weary of having his investigations limited by an elaborate instrument

Now, aged about 43, he reverted to his study of vascular reactions of the skin begun at Hampstead in 1915-16 Within three years his work had opened an entirely new chapter in our knowledge of the behaviour of the peripheral vessels and it was derived largely from observations on the skin of living men Chief amongst the new discoveries was the common pattern of the reaction of the skin to injury which he called the triple response and identified as due to the release from the injured cells of a histamine-like substance The methods successfully used to elucidate the behaviour of normal vessels were next applied to a study of vascular disease, and Raynaud's disease and acrocyanosis soon yielded to the new attack These diseases, previously ascribed to a disorder of the vasomotor nerves were conclusively shown, and by a brilliant use of the experimental method with the simplest apparatus, to be due to a local fault respectively in the digital arteries and small arteries of the skin, an over-reaction to cold Work on arrested blood-flow to the limb revealed the differential paralysis of nerves subserving different functions, work which when correlated with experimental studies of nerve conduction rates by others has added materially to our knowledge of the relationship of nerve-fibre size to function The pain of intermittent claudication was shown to be due to a chemical substance released by muscular contraction, a finding which Lewis was quick to perceive also applied to angina pectoris

The work on obstructive vascular disease led Lewis to his final interest, pain After completing the work on intermittent claudication, he turned to a study of skin pain and showed that this was of one type, easily distinguished by its quality from deep pain, which work from his laboratory again showed to be of one kind irrespective of the nature of the deep tissue from which it arose He demonstrated the intervention of chemical agents not only in the production of cutaneous pain, but also in the widespread tenderness , his experiments forced him to attribute this tenderness to long axon reflexes in fibres not previously known and which he named the nocifensor nerves Work from his laboratory by Kellgren analysed the segmental distribution of pain arising from deep structures, and together they demonstrated experimentally the occurrence of reflex muscular contraction and tenderness from deep somatic structures, thus reopening problems of the first importance in the diagnosis of disease The outbreak of war in 1939 ended this exciting and hopeful research

Lewis was methodical and in his work a pattern was plain First came the period of indecision lasting often for weeks during which he considered what he was going to do and how to set about it , he was moody and fretful and nothing was right , he would pace to and fro, appear in a room, stand silent for a few minutes with lowering brow, and pass on Then, his mind made up, work began and, once begun, nothing was allowed to interrupt its progress, except his regular visits to the wards twice and to the

outpatient department once a week   In his early years he worked extremely hard, all day and often into the night in the laboratory and then for the rest of the night and into the early morning at home analysing and recording results   Sundays and public holidays were grudged as time wasted   This went on for 10 months out of every 12   But for two months he was on holiday and then he would leave medicine entirely behind him and, no less intensely, observe and photograph birds   After his first coronary thrombosis in 1927 the pace slackened a little , he went home about five, and to bed by midnight, and he took up a slightly less exacting pastime, fishing, but the pattern remained the same   Relatively little of his work was wasted , for he was an accurate and penetrating observer and he made concise notes in his own hand on anything he saw that interested him, often laying them aside to be put together at a later date   While the experiments were still in progress he would start entering them up for publication, so that the final paper was often ready within a week or two of the work being ended   The full evidence was published, usually in this journal, a series of papers being often followed by a more general account in one of weekly journals, so that the import of the new work should be conveyed in simple terms to the medical public   Finally he never abandoned a field of work without summarising his own and previous work in a book   The Mechanism and Graphic Registration of the Heart Beat, The Blood Vessels of the Skin and their Responses, and Pain are still the most complete and authoritative works on these subjects, while Clinical Disorders of the Heart Beat, Clinical Electrocardiography, Diseases of the Heart, and Vascular Diseases of the Limbs have won their place in the libraries of countless students and practitioners

Such in brief outline was Lewis's scientific work, told by himself in more than 230 papers and 12 books, work which the world has recognised as having opened out new viewpoints in every field he touched   In his early years, this work was to Lewis all that mattered   It is true that he religiously saw and cared for his in- and out-patients and he taught his students, for these were the duties of his office as physician to University College Hospital   He made few friends, and these chiefly amongst his senior scientific colleagues whose work and judgment he respected   Very few of the young men who came from all countries to work with him and almost none of his students knew anything of the man   But marriage in 1916 to Lorna Treharne James cracked the hard shell of dedication to work alone and released for wider easier friendships his underlying warmth of heart

A further change came after his first coronary thrombosis when in the long convalescence from this illness and away in the tranquillity of the countryside, he had leisure to think over his work and its meaning, and the future   He saw that he might die leaving much work not yet done, and he feared lest the vast mine of knowledge which his pioneering work had begun to open to the world might then be left to close again   He saw that if the

work were to continue after him, it was not enough for him to set the example, but that he must attract recruits to train, and that he must foster a movement to apply the methods of science to the study of disease in man   The plan matured slowly, for Lewis never acted without due consideration, but in 1930 it was launched with his customary vigour   In that year he changed the name of his department to the Department of Clinical Research, and induced the Medical Research Council to make a statement in which they declared their policy of attracting men of ability to clinical research and of maintaining them.   He published in the British Medical Journal an article entitled " Observations on research in medicine , its position and its needs " in which he stressed the difference in outlook and training of those profitably engaged in curative medicine and of those in the advancement of medicine , and made a strong plea for the recruitment of workers and for establishment of posts in the latter field   Finally he established the Medical Research Society, to bring together whole-time workers in clinical research to present the results of their work for mutual criticism and discussion   The new movement did not lack critics , but Lewis was not daunted and returned to the campaign in his Harveian oration of 1933 on " Clinical Science " and in his Huxley lecture in 1935 on " Clinical Science within the University " These, together with two other kindred lectures, he republished as " Research in Medicine " in 1938

A journal was needed for the publication of papers dealing with all aspects of clinical science, and Lewis decided for this purpose to enlarge the scope of Heart and change its name   From 1908 onwards he had been the first and only editor of Heart, its chief contributor, and the mainstay of its scientific repute   After his first illness Lewis, with the agreement of the publishers who were also the owners of Heart, had transferred the responsibility for the appointment of editor to the Medical Research Council His next step, in 1931, was to change the title to one naming more clearly the purpose he had in mind   " Clinical Science " was chosen, for clinical science, first used by Sir James Paget, was the term he had selected as succinctly expressing the aims of the journal and its contributors   Editorial responsibility for the journal was transferred to the Medical Research Society in 1939   Largely through Lewis's efforts, through the munificence of the Nuffield Foundation, and with the generous co-operation of the publishers there seems every prospect of the full ownership of the journal passing into the Society's hands, thus completing the plan which Lewis had entirely conceived and so largely executed.

Lewis took as much trouble with his writing as with his experimental work   Most of his papers were rewritten once or twice before he deemed them finally fit for publication   As an editor he demanded the same high standard of expression from his contributors as from himself, for he held strongly that an editor was responsible to his readers for allowing no looseness of expression, no ambiguity of meaning, no false conclusions, and no redundancy to appear in print   His criticisms, blunt and to the point, were

pencilled on the manuscript and few papers finally appeared in their original form   While such methods were sometimes resented as interfering with individuality in style, most writers were grateful for his tutelage

﹅ In his later years Lewis became intensely interested in medical education   He had always taught his students conscientiously and his ward rounds were models of method in eliciting the clinical facts, and of clear logic, but classes were small because he scorned examination teaching and awed his students   As he grew older he began to unbend   For one year he took a special class on Clinical Science, lectures which he later wrote up in book form   Of this class a substantial number have since made their name in scientific work   Early in the recent war he took charge of all his school's students in their first clinical year who had been evacuated to Cardiff, supervising their whole clinical instruction   He wrote up his considered views on Medical Education, as delivered to the Goodenough Committee, in lecture form in the Lancet in 1943   His "Exercises in Human Physiology" published just before his death, is the first constructive attempt to co-ordinate pre-clinical and clinical education

The exceptional achievements of Lewis during his lifetime were those of no ordinary man   It is true that his father was a man of means, and that poverty never dictated his career , that his wife was a very able woman who with sympathetic devotion lifted many of the cares of family life from him , and that he was fortunate in his early association with men of the calibre of Horsley, Starling and Mackenzie   Similar circumstances have been enjoyed by many men who have achieved little   In the final analysis Lewis's achievements were due to his exceptional ability and above all to his character   He used his hands precisely and with appreciation of material , he was skilled in the use of tools and machines , he wrote when he wished a fair hand , he drew accurately and painted well, though he had no liking for the subtleties of the modern school , his eyes missed nothing, and he could tell details of colouring in a small bird 50 yards away   He had a retentive memory holding details of papers he had read long before   His clear and logical mind, relentlessly distinguished what was fact and what was hypothesis, assembled critically all relevant evidence, then formed its own firm judgment   Those who had the good fortune to work in his department know that even when he was discussing a problem outside his own immediate province, his criticisms were so apt and his suggestions so sensible that he seldom failed to help   Yet it was not so much this high natural ability that distinguished Lewis, as the way in which he used it   He was, as has been mentioned, a tireless worker whose only real happiness lay in achievement   Moreover he always saw, at first mistily, and then ever more clearly the goal at which he was aiming and allowed nothing to deflect him from his purpose   He felt he had a mission in life which was to achieve those things which lay within his ability to achieve and whose value he appreciated though his contemporaries did not   He therefore avoided social intercourse because it wasted time, and made few friends until his

later years when he formed warm attachments with the younger people working in his field , and they had reason to know how affectionate and considerate a friend he could be   He never undertook work which lay outside his central purpose and which he knew others could do as well as he   Except for his diligent service on the Council of the Royal Society and on the Medical Research Council, he avoided committees   He refused to give testimonials to students or, except on very rare occasions, to see patients in whose disease he was uninterested or who were not admitted to his hospital service   When he was busy with an observation or experiment he allowed no interruption, however exalted his visitor , when deep in thought at the lunch table or in the corridor, it was a lesson soon learned that to try to make conversation was to court a look and a few cutting words that soon closed the incident   As a consequence Lewis was often regarded as cold and unfriendly and narrow in his outlook   But those who appreciated the man and his work knew that such was the price that he had to pay for what he had set out to do , and those who gained his friendship and who knew him in his leisure, devoted to his wife and three children, knew a warm-hearted man, whose support once gained was unstinted, and who was a delightful and witty conversationalist on many topics   Outside his work his chief interest was in natural history and in practical matters   He was an authority on birds, having made one of the finest collections of photographs of them in the country, and he knew much of the peculiarities and habits of our common wild animals   To hear him discourse on the principles of conjuring or of billiards was to know that he never developed an interest without the urge to master the subject   In poetry, music, art, drama and philosophy he was not really interested and they occupied little of his leisure   Essentially practical in his outlook Lewis had a shrewd business sense and could drive a hard bargain   All his ventures both scientific and administrative were soundly conceived and soundly executed , what he left was solidly built

Many honours came to Lewis in his lifetime, chief among them the Fellowship of the Royal Society, and its Royal and Copley Medals   But none of them gave him so much satisfaction as to see a job well done, for Lewis was a craftsman in all he did

Lewis had his first coronary thrombosis in 1927, his second in 1935   From these he made an excellent recovery and until the summer of 1944 maintained his usual strenuous life, its leisure spent in trout fishing or gardening with all its share of heavy manual toil, but the late autumn of that year brought the first of a series of attacks of cardiac asthma, to be followed by a pulmonary embolus soon after Christmas   The meaning of these changes was as clear to him as was his conclusion that a life of inactivity and invalidism was unacceptable   Very soon he was once more out of doors and striving for recovery   On the night of March 16th came a last coronary thrombosis and death in the morning   Always consistent, for him it was his best or nothing

G W P

ANERGY IN INFANTS' SKIN TO DIPHTHERIA TOXIN

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IT is now known that in early infancy the Schick test is unreliable as a guide
to the level of immunity to diphtheria   This conclusion, which is of
considerable importance in epidemiology, since it affects the interpretation
of most of the earlier observations on congenital passive immunity, has
been reached mainly from comparisons of simultaneous skin tests on mothers
and their infants—more particularly when these tests have been carried
out in conjunction with titrations of the antitoxin concentrations in the
sera of both   The results obtained by earlier observers, together with those
obtained by us, are given in summary form in the following table

Comparisons of Schick reactions in mothers and infants

	Infants aged under	Mother +ve Infant +ve	Mother —ve Infant —ve	Mother +ve Infant —ve	Mother —ve Infant +ve
Ruh and McClelland (16)	7 days	20	75	4	1
Kuttner and Ratner (11)	14 days	4	39	7	0
Cooke and Sharma (4)	10 days	59	204	34	1
Greengard (6)	14 days	22	45	33	0
Wright and Clark	5 days	66	56	21	2
TOTALS		171	419	99	4

From this table it can be seen that the reactions of mothers and their
newly-born infants often differ, and that the disagreement is due almost
entirely to the failure of the diphtheria toxin to excite a positive response
in many infants of Schick-positive mothers   This lessened skin reactivity,
which in its extreme form has been termed " anergy " by Friedemann (5),
was present in more than one-third of the infants of Schick-positive mothers
examined in these five surveys
   There are no grounds for believing that the anergy shown by these
infants to diphtheria toxin is due to its specific neutralization by circulating
antitoxin, as occurs in older Schick-negative subjects   Several investigations,
notably that of von Groër and Kassowitz (7), have shown that the
concentrations of antitoxin in maternal and cord blood are the same or

almost the same in nearly all instances   Even though a few cases were recorded by these authors in which the antitoxin in the cord serum was higher than that in the maternal, such exceptions cannot account for the fact that positive reactions are almost always uniformly smaller and more transient in infants than in their mothers   It seems almost certain too, that this anergy is non-specific in nature, for its occurrence with the Schick test in newly-born infants is only one example of the irresponsiveness of their skins to certain kinds of irritants   Other instances of anergy have been demonstrated for streptococcal erythrogenic toxin (Dick test) by Cooke (3) and by Paunz and Csoma (14), and for quinine, turpentine, iodoform and other substances by Adelsberger (1)

In spite of its interest in relation to the general problem of the reaction of the skin to injury, and its significance in estimating degrees of immunity to diphtheria in infancy, little attention has been given to the factors underlying this anergy   The present paper records the results of some experiments that we have made to find out in what ways the reactions caused by various irritants and by histamine in the skins of newly-born infants differ from those in adults

The reactions of infants' skins to histamine and other factors causing immediate responses

If histamine acid phosphate (1 1000) be pricked or injected intra-dermally in 0 1 c c doses into the skins of newly-born infants, the triple response described by Lewis (13) can be seen to develop in exactly the same way as in adults   With smaller amounts of the drug, essentially similar, but less intense, reactions develop   If the infant's skin is naturally red, the outer edges of the flare are often not easily distinguished, but in pale infants, who remain quiet, the flare may attain a brilliance and distinctness quite unusual in older subjects   There are, however, two minor points of difference which distinguish the reactions in newly-born infants   (i) their relatively greater size, and (ii) their more rapid rate of fading   Nevertheless, in spite of these differences, it is clear that the minute vessels of the skins of infants are capable of precisely the same responses to histamine as those of older subjects   Anergy to diphtheria toxin and other substances cannot, therefore, be due to insensitivity of the skin vessels to histamine

Nor is the anergy due to any inability of the cells in infants' skins to release histamine-like substances on injury   Typical immediate responses can be evoked by chemical irritants, such as dilute caustic soda, and by physical agents, like minor mechanical trauma   Further, we have found that the local sensitization reaction described by Prausnitz and Küstner (15) and even better, its converse, the reversed passive skin sensitization described by Wright and Hopkins (21), can be elicited with horse serum and human anti-horse serum as readily in the skins of newly-born infants as in those of

adults   With these reactions, however, as with those from histamine, the flare and wheal disappear sooner in the infants

From the ease with which these reactions are evoked, it is clear that anergy cannot be ascribed to any inability of the infants' skins to release histamine-like substances on injury   Nor would this be expected from the observations of Harris (8), who found that the skins of premature and newly-born infants contain relatively large quantities of histamine-like substances

The reactions of infants' skins to irritants causing delayed responses

Although the direct responses described above tend to be more transient in newly-born infants, their intensity at their height is fully as great as that in older subjects   The phenomenon of anergy is essentially associated with the delayed type of response to injury, which only begins to appear after a lapse of several hours and reaches its culmination much later still   The cause of the latency is not known, though we have provided evidence (23) that in the Schick test more than 12 hours must elapse before the toxin becomes fully fixed by vulnerable cells, and that a still longer interval is needed for this fixed toxin to injure those cells sufficiently for them to release vaso-dilator substances in amounts adequate to produce visibly recognizable reactions   Were diphtheria toxin to be removed from the site of injection by cutaneous vessels more readily in young infants, the loss of a greater fraction of the test dose during this long latent period before fixation would lessen the intensity of the ensuing reaction   Although the fluid blebs following intradermal injections disappear more quickly in infants than in adults, this difference cannot wholly account for the anergy, because the skins of newly-born infants are also less responsive to the physical stimulus of ultra-violet light—another agent capable of evoking delayed responses   This observation was first made by Vollmer and Frankenstein (20), we have repeated it upon eleven infants varying in age from birth to two months, and our findings are in agreement with theirs   An exposure to a mercury-vapour lamp at a distance of 30 cm , through three apertures, for 1, 2 and 3 minutes, gave consistently graded reactions in the skins of adults and children aged from six weeks upwards   Below this age, however, the resulting erythema is much less intense, and is often scarcely discernable even after the longest exposure

Discussion

Since the immediate responses to irritants and to histamine in newly-born infants and in adults exhibit only superficial differences, they would seem to depend upon the same mechanism at all ages   On the other hand, the delayed responses of diphtheria toxin and ultra-violet light are evoked readily in older subjects, but with difficulty in newly-born infants   This

difference would seem to imply either that the delayed response depends upon some local vaso-dilator mechanism which differs essentially from that associated with histamine-like substances and which is not fully functional in the skin for some weeks after birth, or that during delayed responses the vaso-dilator substances concerned are released more slowly, or removed more rapidly, in the skins of infants, so that they fail to attain the concentration needed to evoke a characteristic response

On the possibility of the first alternative, we have no further evidence to bring forward , on the second, certain relevant information is available

Observations on the rate of development of immediate responses show that they develop just as rapidly in the skins of newly-born infants as in those of older subjects   Consequently, there is no reason to believe that, if histamine-like substances are also responsible for delayed reactions, they would be released more slowly in infants' skins   On the other hand, such infants' skins do possess structural features which might well promote the more rapid removal both of any injected substance and of any vaso-dilator substance released locally   Ruh and McClelland (16) found that greater pressure is required to deliver the test dose of toxin intradermally in infants than in adults, and that the resulting bleb is smaller and disappears more quickly   Our own observations are in agreement with theirs   Kuttner and Ratner (11) also suggested that anergy is due to the prompter removal of the toxin from the site injected

The blood vessels of the human skin at various ages have been examined by histological and injection methods (18, 19), and by transillumination (9, 10, 17)   The progressive changes in the skin circulation in the first few weeks of neonatal life are particularly clearly shown in the illustrations published by Schwalm   At birth, the interpapillary down-growths of dermis have scarcely developed, so that little tissue separates the sub-epithelial basement membrane from the rich network of minute vessels in the corium (2)   Within a few weeks, the epidermis thickens, and an interdigitating zone formed by epithelial down-growths and capillary-containing papillary up-growths becomes more conspicuous   The formation of this zone thus removes the basement membrane, and therefore any fluid injected intradermally, to a greater distance from the main group of underlying vessels, and especially from the relatively permeable venous plexus

To ensure that these earlier observations were pertinent to the present discussion, we compared the histological appearances of the skins of six infants, aged from birth to 14 days, with those of ten adult women   The specimens were removed *post mortem* from the sites used for Schick testing, and were stained with hæmatoxylin and eosin, and by Mallory's connective tissue method   In the infants' skins there were many large sinusoidal vessels, with walls formed by a single layer of endothelium   These vessels

lay close beneath the basement membrane of the epithelium, and were thus very close to the layer of the skin that would receive the bulk of any fluid injected intradermally In the skins of the adult women, the vessels were much fewer in number, and generally more deeply placed Those in the upper layer of the corium were capillary in character, and only occasional vessels in the deeper layers were in any way comparable in size with those seen in the infants , from the muscular structure of their walls, these larger vessels seemed to be small venules No attempt was made to measure the vascular absorptive areas in the skins at the two ages, but it is clear that that of the infants is much the more extensive

Part of the anergy to diphtheria toxin in early infancy may thus be attributable to an unusually rapid escape from the site, of the substances concerned in the various stages of the reaction Ruh and McClelland specifically mention the removal of the toxin itself, but if this vascular explanation is to suffice for delayed reactions of all kinds, the vaso-dilator products of cell injury must be removed at least as effectively Even were the latter not so diffusible as histamine acid phosphate, the intensities of whose action were found by Lewis and Grant (12) to differ considerably in cool and warm skin, they are hardly likely to be less diffusible than diphtheria toxin which has a molecular weight of about 70,000 Operating in both the first and second ways, the highly developed vasculature would lessen the intensities of the delayed responses caused chemically by toxins and physically by ultra-violet light in newly-born infants' skins

SUMMARY

The low reactivity of newly-born infants' skins to diphtheria toxin is not due to any insensitivity to histamine, or to inability to exhibit the typical triple response on injury Since similar anergy is found in other forms of delayed response at this age, such as that produced by ultra-violet light, the mechanism underlying these responses may differ from that concerned with immediate reactions, and may not become fully functional until some weeks after birth It seems likely, however, that the lesser skin reactivity seen in delayed reactions in infants is partly due to the relatively rich and superficial vasculature of their skins, which would facilitate the escape both of injected substances and of vaso-dilator products of injured tissues

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ANATOMICAL CHANGES IN THE LIVER AFTER TRAUMA

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THE descriptions of anatomical changes after trauma have been largely
vitiated by lack of adequate clinical details , " shock " has been thought to
be a sufficient clinical description of the often multiple derangements of
function following such different lesions as lacerations with internal and
external hæmorrhage, severe fractures with fat embolism or muscle necrosis,
crush injuries, burns or visceral damage   The purpose of this publication
is firstly to present an account of the anatomical changes found in the liver
following crushing injury, severe fracture and several other types of trauma
and secondly to correlate these changes with the functional aberrations
observed during life

Material and methods

Material was available from 24 cases dying after crushing injury with
prolonged compression and from 18 cases dying after severe injury not
involving prolonged compression, mainly fractures   All cases except
No 26 were known to have muscle necrosis , in cases 1-25 this was
considerable (about a kilogramme)   A much smaller amount was necrotic
in cases 25-42   Ten of each group were personally observed throughout
most of the remainder were seen clinically during the latter part of their

course and some previous data were obtainable   Liver was fixed in 5%
formol-saline and Helly's fluid , in nine cases, formol-saline was injected
into the liver within an hour of death   In 21 instances, material was
obtained for histological examination from both right and left lobes, selecting
an area on the convexity of each lobe about 3″ from the lower border, or
the " prefixed " area nearest to this   In two cases, Dr Sherlock obtained
material from the right lobe by needle aspiration immediately after death

Paraffin sections, 6 μ thick, were obtained by hæmatoxyln and eosin
and by a Mallory method using McFarlane's (17) and Wigglesworth's (26)
modifications   Frozen sections, 15 μ thick were stained by Sudan IV
No examination for glycogen was made

Mitoses were counted in 100 successive high power fields, and are
expressed as a number per 1,000 liver cells , an average figure was taken
for the total number of cells per high power field   All phases were counted
except that in which the nuclear membrane was intact   The distribution
of mitoses in relation to central veins, portal tracts and areas of necrosis
was obtained by drawing a lobule from alternate serial sections by projection,
marking in the mitotic figures and checking each one by direct vision under
a 1/6 objective   The mitoses, vessels and areas of necrosis were then marked
on the drawing with a radio-opaque suspension of lead dichromate in gum
arabic , successive drawings were then superimposed and a roentgengram
taken of the whole   Lobules were selected in which there was little change
in shape or size through the thickness covered by the serial sections

RESULTS

Mitotic activity

Mitotic activity was seen in 8 of the 24 crush injury cases and in 2 of the
18 non-crush cases   (Tables I and II)   In material from routine hospital
autopsies fixed at a variable time after death, from biopsies fixed immediately
after removal and from the remainder of each of the present groups, no
mitoses were seen despite prolonged search through each section   In three
cases, mitoses were very numerous, averaging two per high power field in
Case 1 (Fig 1), with one field showing 10 mitotic figures   Nuclei in
premitosis, that is with coiled chromatin but before rupture of the nuclear
membrane, were also numerous

The distribution of mitoses in the lobule was studied in two cases
Case 1 showed no necrosis and had had no fall in blood pressure below
100 mm Hg   in the right lobe, Fig 3A, mitoses are seen scattered uniformly
through this lobule, sparing perhaps the immediate periportal cells , the
left lobe, Fig 3B, however, shows a greater frequency in the area between
two central veins   Exactly the same pattern was seen whether the
superimposed drawings were aligned through the two central veins, through

the two portal canals or through one portal canal and one central vein
Fig 2 shows the midzonal distribution of mitoses in a lobule, Case 2, in
which there was no necrosis   Fig 4, a lobule with necrosis from the same
section, shows that mitoses surround the necrotic area, and, as in Case 1,
tend to be most numerous on a line joining two central veins   In other
cases with fewer mitoses, these still seemed to occur with great frequency
in the immediate neighbourhood of the midzonal or paracentral necrotic
scars where these co-existed (Cases 5, 7, 30 and 31) and in midzonal areas
in cases where there was no necrosis (Cases 3 and 4)   Case 15 must be
considered separately from the others since she had suffered a small laceration
of the liver   there was massive necrosis at the site of laceration and central
necrosis in some of the lobules immediately surrounding it   Mitoses were
more frequent near the necrotic area, (3 2 per 1,000 cells at 0 5 cm from the
necrosis) but beyond this there did not appear to be any significant change
in frequency, counts being 1 8, 1 0, 1 3 and 1 4 mitoses per thousand cells
at 1, 1 5, 2 and 2 5 cm away respectively   Unfortunately, no histological
examination was made of more distant portions of liver and it is not therefore
possible to say that this was entirely related to the local trauma

Relation to necrosis

Mitoses occurred without necrosis in four patients (Cases 1, 3, 6 and 4),
necrosis without mitoses in nine patients (Cases 14, 25, 26, 27, 28, 29, 32,
40 and 42)   None of them showed both a survival time over 5 days and a
death to fixation time of under 24 hours   Necrosis and mitoses occurred
together in six cases

Relation to autolysis

The mitoses in the present material were usually of normal configuration,
with an angle averaging 56°, although in cases fixed late metaphase
predominated (e g , constituting 90% of the figures in Case 3) and
abnormalities made recognition slightly more difficult   Occasional mitoses
were seen in tissues fixed 23 (Case 31) and 36 hours (Case 7) after death
they were relatively numerous in Cases 3 and 6, fixed 16 and 18 hours after
death   It is not known how rapidly such mitoses disappear from the liver
after death, but there is probably a wide variation depending on factors
such as temperature   Mitoses in a sarcoma have been shown to survive
unaltered in number for 24 hours after death, whether the tissues were
kept at 37°, 20°, or 0° (9)   in chilled tadpoles, mitosis is arrested in
metaphase (12)   Of the cases showing necrosis without mitoses, only
three had been fixed within 24 hours of death (Case 42 fixed at 7 hours,
Case 32 fixed within one hour and Case 40 fixed at 0 3 hours)   These cases
lived for 4 7, 1 7 and 1 1 days after trauma respectively

TABLE I

CRUSH SYNDROME CASES ||

Case No	Hours Burial	B P fall and Duration	Hb g %	Transfusion ‡ (litres)		Cause of death	Survival time (days)	Death to fixation time (hr)	Mitotic index *		Necrosis grade (5—0)	
1	3 5	None	17 1	None		Uræmia	7	7	5 2 (L)	9 2 (R)	0 (L)	0 (R)
2	5	Below 90, 7 hr	20 3	P	3 0	Uræmia	7 5	3	5 7†		3	
				B	0 6							
3	10	None	20 7	P	1 1	Uræmia	6	16	3 5		0	
4	8	75 on entry	—	P	1 1	Uræmia	7	—	2 5		0	
5	9	Below 90, 2½ hr	12 2	P	2 7	Uræmia	7 7	2 5	1 9§		2	
				B	1 1							
6	6 2	Below 90 at 10 hr	—	P	3 2	Uræmia	7 0	18 2	0 6 (L)	1 0 (R)	0 (L)	0 (R)
7	11	Below 90, several hr	—	P	1 1	Uræmia	7 7	36	0 1		4	
				B	0 6							
8	9 5	60 on entry	15 2	P	0 8	Uræmia	9	4 5	0 (L)	0 (R)	0 (L)	0 (R)
9	?	? None	—	None		Uræmia, Alkalosis	7	—	0		0	
10	12	90 for 3 hr	—	None		Uræmia	7	—	0		0	
11	6	Below 90, 13 hr	13 8	P	2 2	Uræmia	6	12	0		0	
				B	0 6							
12	12	—	22 0	P	1 9	Uræmia	5 5	—	0		0	
				B	0 6							

13	4	—	—	P B 2 2 / 0 0	Uraemia	4 2	2 4	0	0	0
14	0	—	—	B 1 1	Uraemia	4	—	0	0	2
15	4	90 for 24 hr (v low once)	20 8	P 3 0	?	3	8	1 4	1 4	Liver tear
16	6 6	—	—	None	Necrosis of gut+uraemia	2	—	0	0	0
17	12 6	—	—	P 1 7	?	0 5	16	0 (L) (R)	0	0
18	10	Below 70, 5½ hr	10 2	P 2 4	?	0 4	6	0	0	0 (L) 0 (R)
19	7	Below 90, 4 hr	10 0	P 3 0	?	1 3	47	0	0	0
20	1 2	—	—	P 0 5	Uraemia	6	72	0	0	0
21	4	Below 90 at 6 hr	20 8	P 1 3	?	1 0	13 6	0	0	0
22	1 7	Below 90, 1½ + 4 hr	13 0	P 0 6	"Shock"	0 4	0	0 (Rt lobe aspiration)	0 (Liver aspiration)	0
23	8 6	Below 90 for 7 6 hr	—	P 0 6	Fat embolism	0 3	0 1	0 (L) 0 (R)	0 (L) 0 (R)	0
24	0 4	Not below 90	17 6	P 2 1	Fat embolism	1 4	117	0	0	0

* Mitotic figures per 1,000 cells, 0 = no mitoses seen in slide
† Pancreas 1 6 : mitoses also in kidney
‡ P = plasma or serum   B = blood
§ Mitoses in kidney and thyroid
‖ Plasma bilirubin below 1 2 mg % in Cases 2 and 3, and 1 2 mg % in Case 15   Other plasma was normal in colour

TABLE II

CASES WITHOUT PROLONGED COMPRESSION

Case No	Injury	Duration of B P fall 90 mm or below	Transfusion (litres)	Cause of death	Survival time days	Death to fixation time (hr)	Mitotic index	Necrosis grade (5—0)
25	Fract both legs and pelvis	0 hr	P 0 6 / B 3 4	Uraemia	3 5	60	0	3
26	Cerebral cortical opn	"Low most of the time"	—	Uraemia	2 75	—	0	4
27	Fract pelvis and tibia gut ruptured	4 + 27 hr	P 1 1 / B 1 7	Uraemia, Peritonitis	8	46	0	4
28	Traumat arm amputation	? "Not below 90"	P 0 6 / B 2 8	Uraemia	7 5	34	0	4
29	Thigh pulped, fract pelvis, torn artery	4 + 4 + 13 hr	P 1 7 / B 2 4	?	1 7	33	0	3
30	Kidney rupture, necrotic rectus abdominis	?	B 2 4	Uraemia	6 8	16	1 8 (L) / 0 78 (R)	3 (L) / 1 (R)
31	Fract pelvis, spine, lt leg	About 24 hr	P 1 7	Uraemia	6 2	23	0·45 (L) / 0 05 (R)	4 (L) / 2 (R)
32	Traumt foot amputation fract scapula	5 + 7 + about 12 hr	P 3 9 / B 3 1	Uraemia ?	1 7	1	0	3 (L) / 0 (R)

								(L) (R)
33	Traumat amp n both legs	1 + 34 hr	P 0 3 / B 1 5	Fat embolism	1 7	0 2		0	0 0
71	Multiple fractures and head injury	4 hr	None	"Shock"	0 2	0 5		0	0 0
75	Gross muscle laceration, ruptured ileum	4 + 7 hr	P 1 7 / B 3 4	?	1 2	0 1		0	0 0
90	Head injury, fract femur	6 hr	None	Head injuries	0 3	0 7		0	0 0
77	Traumat amp'n of forearm	None	None	Aspiration of vomit	0 3	0 7		0	0 0
38	Fract calcaneal, forearm, pelvis	20 hr	P 1 7 / B 0 6	Fat embolism	0 8	0 2		0	0 0
39	Fracture of arm	1 hour before death	B 1 7	Fat embolism	1 6	1 6		0	0 0
40	Fracture pelvis, rectal tear	6 + 7 hr	P 4 5 / B 1 7	Gas gangrene	1 1	0 3		0	4 (L) 6 (R)
41	Fracture pelvis	4 hr	B 1 2 / P 1 7	Uremia	4 3	0		0	0 (L) 0 (R)
42	Fracture tibia and fibula	?	None	Uremia	4 7	7	0 (L) 0 (R)		2 (L) 1 (R)

Plasma bilirubin 1 0 mg % in Cases 25, 41 and 42, 1 8 mg % in Case 70, 5 0 mg % in Case 32 and 2 0 mg % with haemoglobinemia in Case 40. Case 27 was jaundiced

Relation to survival time

No mitoses were seen in cases dying under six days from time of injury except in Case 15 surviving for three days and associated with rupture of the liver Of 15 cases dying after six days or more from the time of injury, 6 showed no mitoses (Cases 8, 9, 10, 11, 28 and 27) Of these 6, however, only two were known to be fixed within 24 hours of death (Cases 8 and 11), and neither of these showed necrosis

Table 3, summarises the above analysis of the relation of mitosis to the two of the three variable factors, necrosis, autolysis and survival time The facts may be explained by three assumptions that mitoses tend to disappear after 24 hours autolysis that they appear in general only after six days from the time of injury, the exception being a case with direct liver trauma, and that they may be present with or without liver necrosis,

TABLE III

Survival Period	Less than 6 days		6 days or more	
Death to Fixation Time	Less than 24 hrs	24 hours or more	Less than 24 hrs	24 hours or more
Mitosis	1	0	7 (1 not known)	(1) known)
No mitosis	18 (4 not known)	3 known)	2 (2 not known)	3 known)

but that they tend to occur in that portion of the liver lobule which in this series of cases, shows necrosis No relationship can be seen to type of medication, amount of transfusion, or duration of blood pressure fall Nor is it possible to relate the amount of muscle necrosis with mitosis, within those 10 cases with a survival greater than six days and a death to fixation time of less than 24 hours, two showed no mitosis (Cases 8 and 11) and yet both these had muscle damage of over one kilogramme in contrast Cases 30 and 31 had a very much smaller amount of necrotic muscle but showed mitoses

Necrosis

As may be seen from Fig 5, necrosis when of severe degree spared only the periportal zone when of lesser degree, it affected only one or two quadrants of the lobule and appeared midzonal, (sparing the cells round the central vein) or sometimes paracentral In many cases it started from one segment of the central vein and stretched across towards the corresponding segment of the vein in the neighbouring lobule Necrosis was thus grouped round certain portal tracts, marked A in Fig 6, but no

anatomical lesion could be detected in them In cases with midzonal necrosis, on the anoxia theory, the intact cells surrounding the central veins must be supplied with nutriment from the portal tracts, marked B in Fig 6, through the quadrants of the lobule opposite to the necrosis Thus, portal tracts surrounding a lobule may have a potential territory extending beyond the central vein As the blood supply may be relatively poorer from some portal tracts than from others, this would leave a midzonal area with precarious nutrition.

Necrosis was more marked in the left than in the right lobe in the five crush cases where this was investigated In Case 32, having clinical jaundice and 5 9 mg bilirubin per 100 c c of plasma, a liver aspiration at death showed no abnormality at autopsy, gross necrosis was seen between the central veins of lobules in the left lobe, Fig 6 the right lobe showed no such change Since, in those cases where the two liver lobes were not separately investigated, the single piece came usually from the right lobe, it is possible that several of these other 12 " crush " patients may also have shown necrosis in the left lobe Small degrees of necrosis demonstrated by subsequent histological examination could not be detected macroscopically by skilled pathologists

The earliest degenerative change was in Case 25, with crushing injury, dying, 8 5 hours after release, with restored blood volume and increased venous pressure The liver showed eosinophil globules in the space between the liver cells and sinusoid wall, Fig 7 no necrosis was seen There were also small intracellular, hydropic, non-fatty vacuoles somewhat similar to those described by Kritzler (15) and others (23) in anoxic conditions ; unfortunately, no material for glycogen demonstration was taken The earliest necrotic change seen was 24 hours after injury in Case 40 with fractured pelvis and low blood pressure due to gas gangrene The injured cells showed dense eosinophil cytoplasm and pyknotic distorted nuclei A later stage with further nuclear degeneration had occurred by 1 7 days (Cases 29 and 32) and 2 7 (Case 26) days after injury Another patient dying at 1 7 days after injury, with a B P below 90 for 34 hours due to fat embolism, showed only minor changes such as shrinkage of cell columns in the centre of the lobule, together with dark staining of the nuclei (Case 33) By 3 5 and 4 7 days after injury (Cases 25 and 42) the cells in the centre of the necrotic area had swollen, while those in its periphery had ballooned out, still retaining a central pyknotic nucleus (Fig 8) This vacuolation was hydropic, not fatty and was maximal at this time , patients dying later (Cases 2, 30 and 31) showed only rarely such a vacuolated cell enmeshed in the central area of collapse, with capillary engorgement and occasionally pigment masses Similar cells have been described by Cameron and Karunaratne (5) on the periphery of the central necrotic area 24 hours after carbon tetrachloride injection in the rat They had disappeared by three days

in experimental animals occurs in man , the detailed distribution of pathological changes within the liver has in man only rarely been investigated (25)

The extraordinary increase in mitotic activity observed is not a reaction to liver necrosis, since mitosis was observed without necrosis, but it may be a sequel of reparable damage   The sudden appearance of mitoses coincides in time with the peak of Cuthbertson's " post traumatic metabolic response " (7) but correlation with this in individual cases was not possible since patients who had survived until the 5th or 6th day died of uræmia, retaining most of their metabolic products   It is suggestive, however, that no mitoses were seen in patients dying before the sixth day, except in a case with local liver injury, yet almost exactly similar injuries apart from the actual cause of early death, often an incidental occurrence such as fat embolism was sustained by those patients dying early without mitoses as by those dying after the sixth day with mitoses   Furthermore in two cases liver mitoses were associated with increased mitoses in other organs (kidney and pancreas, Case 2, kidney and thyroid, Case 5)

A similar lag in the appearance of mitoses has been found by Glucksmann and Spear (12)   When tadpoles were starved, mitoses disappeared   following the resumption of feeding, mitoses appeared again only after a latent interval of five days   Such a phenomenon occurs also in wound healing   in corneal wounds during the process of epithelial repair the frequency of mitoses falls off to one half the normal, returning to normal on the fourth day and reaching a peak of nearly twice normal on the fifth day (1)   The peak in mitotic activity of rat liver occurs at 48 hours following chloroform necrosis (10), and at about 3 days following carbon tetrachloride (5) necrosis   In the guinea-pig a large increase of mitoses occurs 3-4 days after partial hepatectomy or ligation of the hepatic artery (19)   It is suggested, therefore, that both mitosis and necrosis are reactions of the liver to injury produced during the phase of blood volume depletion and that other tissues may also show the effects of such injury, including the kidney and the pancreas   It seems possible also that the mitotic increase might follow other types of injury than those dealt with in this series, but that only those who develop uræmia (as a result of muscle necrosis) come to autopsy at a time when mitosis is markedly increased   If death had not occurred, liver damage would have been suspected clinically only in two patients with jaundice   the remainder showed no gross signs and no discolouration of the plasma

SUMMARY

A high proportion of livers from cases of crushing injury and of skeletal trauma from other accidents show necrosis of the central or midzonal cells of the lobule , this appears histologically to date from the time of injury   Necrosis occurs usually in the most severely injured, judged by blood pressure readings and the amount of transfusion fluid given and probably results

from restricted blood and oxygen supply to the liver   The distribution of necrosis within the lobule may be explained by differences of blood supply from the surrounding portal tracts   The left lobe was affected more than the right in all cases where this was investigated

Mitoses were seen in 7 out of 9 patients dying six days or more after the injury, whose tissues were fixed within 24 hours of death, but not in patients dying before this time (except one with direct liver damage) * The distribution of these mitoses within the lobule was investigated   the findings suggested that these mitoses are also related to functional damage to the organ sustained through the circulatory disturbances immediately following injury

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* Since this paper was accepted for publication, material from six further crush syndrome cases and other non-crush cases has been seen, which fits in with the above conclusions, with one exception   this was a crush case, surviving for 2 5 days, in whose liver 1 4 mitoses per thousand cells were seen , there was no necrosis

Fig 1   Case 2   × 500   Hæmatoxylin and eosin   6 mitoses
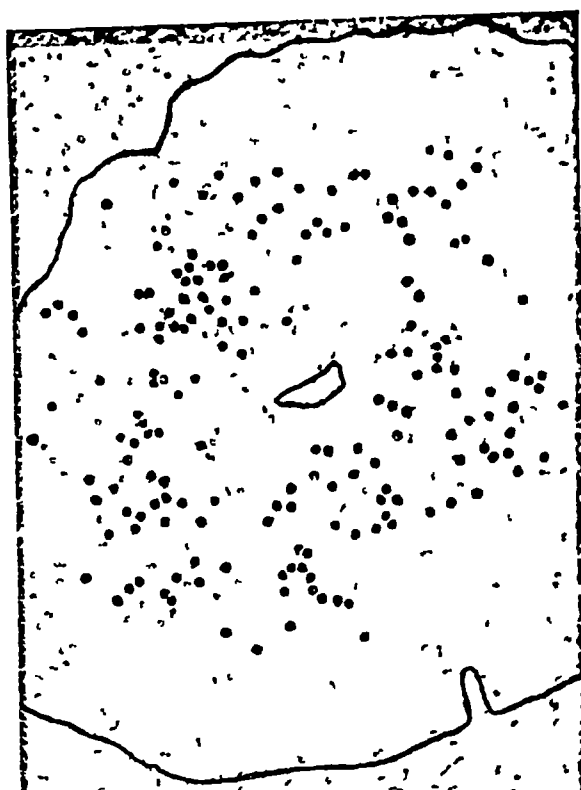
Fig 2   Case 2   × 95   Hæmatoxylin and eosin   Superimposition of mitoses in 16 alternate serial sections from a lobule without necrosis showing midzonal distribution

a   *b*
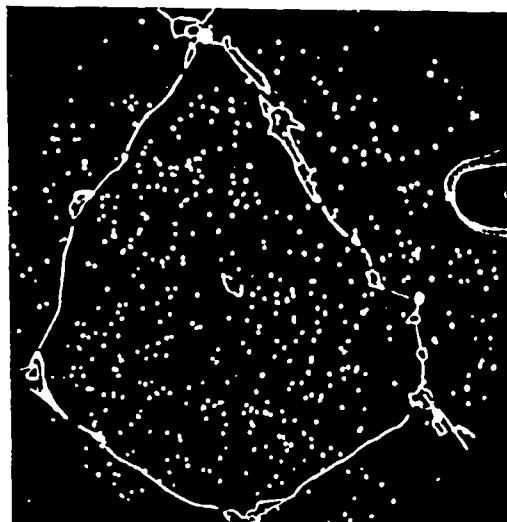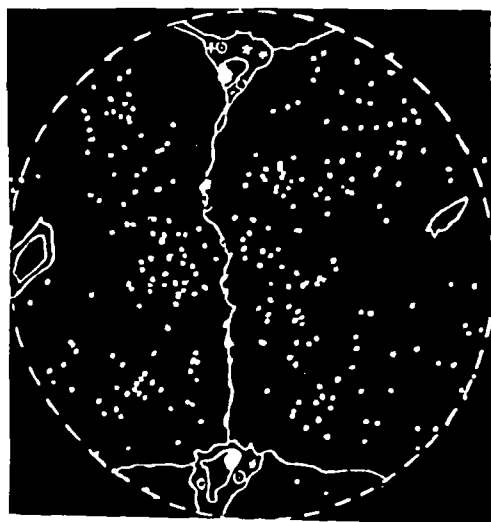
Fig 3   Case 1   × 45   Distribution of mitoses within the lobule, based on 16 superimposed serial sections   (*a*) from right lobe   (*b*) from left lobe   Note even distribution in (*a*) with slight relative sparing only of periportal zones   (*b*) shows the same with a concentration of mitoses in the area between two adjacent central veins

Fig. 1 Case 2 ×90. Haematoxylin and eosin. (a) Superimposition of main vein and preservation from a hepatic vein ... with midzonal necrosis. Veins occur in mid and main region on borders of a zonal area. They are main region at towards a vascular area... central vein passing through the border of the lobule which shows least degeneration mid (bottom). (b) Mid vein foreimpose a ...
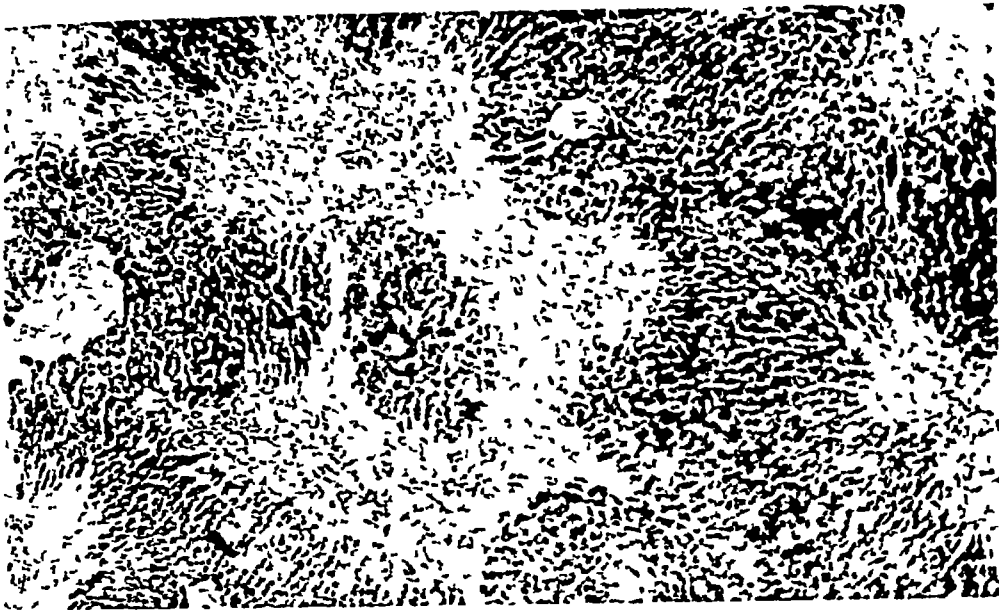
Fig 5 Case 31   °9   Hæmatoxylin and eosin   Left lobe of liver showing extensive necrosis
in periportal zones   The right lobe shows very slight midzonal necrosis
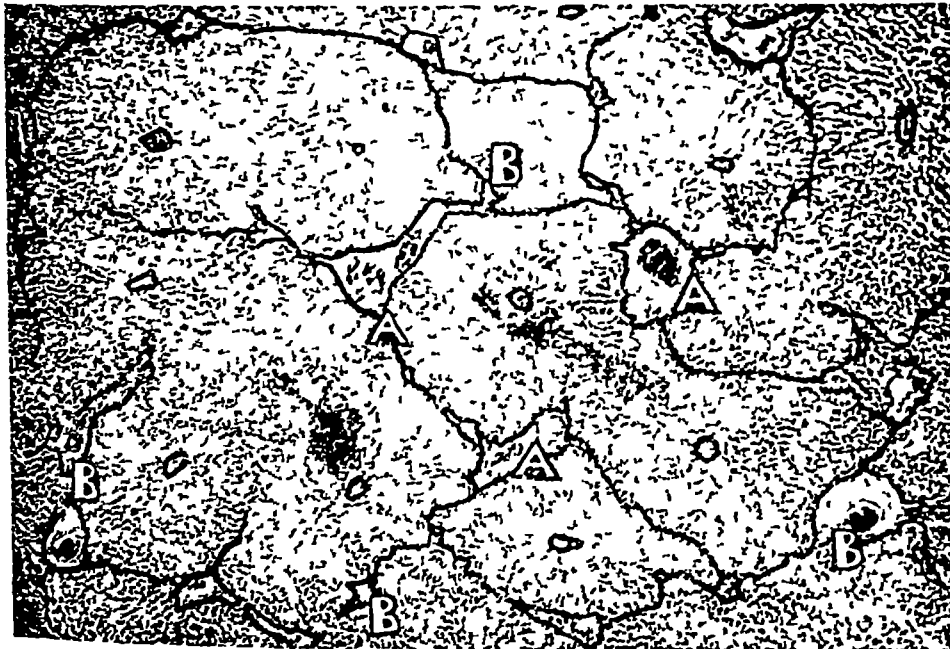
Fig 6 Case 32 × 45   Hæmatoxylin and eosin   Areas of acute necrosis are shaded   lobular
outline and central veins outlined in ink   Note necrosis in neighbourhood of portal tracts
marked A ' extending between central veins in midzone   it spares central liver cells
which are supplied from portal tracts marked   B '

EFFECTS OF VENESECTION IN LOW OUTPUT HEART FAILURE

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It is well known that venesection produces clinical improvement in some cases of congestive heart failure It is usually stated that venesection relieves congestion, increases vital capacity, relieves dyspnœa and cyanosis, and decreases the work of heart (5)

Venesection certainly reduces venous pressure as measured in a peripheral vein (1), but the arm-to-tongue circulation time may remain unchanged Some of our own earlier data with large bleedings are shown in Table I

The central problem has been the effect on the output of the heart Using the difficult acetylene technique, Resnik, Friedman and Harrison (9) found a slight fall of cardiac output after removal of about 500 c c of blood in 3 cases, and from this they argued that the work of the heart was decreased In a study (7) of the action of digitalis on cardiac output and right auricular pressure by the technique of cardiac catheterisation, control observations were made on the effects of simple mechanical lowering of right auricular pressure This measure, effected by inflating cuffs on the thighs to about 80 mm Hg , resulted in a rise of cardiac output in cases of congestive failure with a low initial cardiac output The trapping of blood distal to congesting cuffs involves factors other than a moderate reduction of venous pressure It was thus desirable to study the effects of greater venous pressure reductions following venesection, and the results of this further investigation are here reported

Material and methods

The patients studied had congestive heart failure from valvular, hypertensive and ischæmic heart disease Such cases may be conveniently referred to as " low output heart failure," since initial cardiac output is usually well below the normal level for each individual This term

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TABLE I

*Data from cases of congestive heart failure bled large amounts   Venous pressure was measured
in an antecubital vein, circulation time by the decholin method, and hæmoglobin by a photo
electric method   Measurements were made directly after the end of venesection*

S R = sinus rhythm

Diagnosis	Time	Venous pressure, cm saline above sternal angle	Vital capacity litres	Circulation time, secs	Hb %	Amount venesected c c
Ischæmic heart disease S R	Before After	+15 0	1 7 1 9	53 70	10S 94	1080
Hypertension S R	Before After	+ 3 — 1	72 85	21 21	85 76	1000
Ischæmic heart disease complete heart block	Before After	+ 5 — 1	82 1 2	33½ 33½	100 3 98	1250
Cor pulmonale S R	Before After	+19 + 3 5	1 03 1 2		70 72	1800
Hypertension S R	Before After	+ 8 + 3 0		42 40	103 96	950
Hypertension S R	Before After	+24 +10		61 65	110 98	1080
Aortic incompetence S R	Before After	+15 + 3		•	95 78	1080
Cor pulmonale S R	Before After	+15 + 2			104 99 5	1500
Syphilitic aortic incompetence S R	Before After	+ 9 0			102 95	1100
Mitral stenosis Aur fib	Before After	+11 + 2			98 04	800

distinguishes them from another group where initial output is higher than
normal (4, 6, 10)

Patients were propped up in bed with the trunk elevated 45°   Cardiac
output and right auricular pressure were measured by cardiac catheterisation
The time taken for venesection was from 5 to 40 minutes

TABLE II

Effects of Venesection

Case No	Reference No	Age and Sex	Diagnosis	Time	Right auricular pressure cm saline above sternal angle	Heart rate	Cardiac output lit/min	Blood pressure mm Hg	Amount bled c c	% change in total peripheral resistance	% change in cardiac work
1	6 155	F 60	Hypertension S R	Before After	+15 — 3	88 84	2 6 4 3	242/160 220/112	800+ cuffs	—17	+36
2	7 1	M 57	Hypertension S R	Before After	+26 + 6 5	100 100	2 4 3 0	195/150 195/128	600+ cuffs	—17	+25
3	8 35	F 52	Hypertension Mitral stenosis Aur fib	Before After	+21 + 1	88 88	1 6 2 5	248/140 240/120	1050	—10	+45
4	7 45	F 56	Mitral stenosis Aur fib	Before After	+24 + 8		2 5 3 1	130/80 112/70	430	—31	+ 8
5	7 19	M 52	Hypertension S R	Before After	+23 + 5		3 1 3 9	190/170 175/132	950	—31*	+15*
6	7 55	M 72	Hypertension S R	Before After	+16 + 2	104 86	2 4 3 0	158/100 150/90	700	—26	+16
7	7 5	M 61	Ischæmic heart disease S.R	Before After	+14 + 2 5	112 110	2 2 3 6	128/100 106/86	500	—18	+40
8	6 17	F 64	Hypertension Aur fib	Before After	+11 + 7 5		3 1 4 7		550		
9	5 7	M 57	Hypertension S R	Before After	+12 + 3	82 80	2 1 2 35	110/75 110/74	420	—11	+10
10	7 9	M 50	Ischæmic heart disease Complete heart block	Before After	+10 5 + 4 5	26 27	3 4 4 7	156/70 154/70	cuffs	—27	+38
11	7 15	F 65	Hypertension S R	Before After	— 2 5 — 8	90 92	5 4 5 5	202/112 176/98	550	—14	—12
12	7 17	M 32	Hypertension S R	Before After	— 2 5 — 5 0	108 128	4 7 3 3	270/166 214/156	500	+20	—40
13	6 73	M 74	Ischæmic heart disease S R	Before After	— 3 5 —10	72 80	3 4 2 4	130/70 110/80	200+ cuffs	+35	—32

S R = sinus rhythm

* = calculated from systolic blood pressure

The following formulæ were used for calculating percentage change in total peripheral resistance and cardiac work

"Mean" arterial pressure $= \dfrac{\text{systolic} + \text{diastolic B P (mm Hg)}}{2}$

Total peripheral resistance (in arbitrary units) $= \dfrac{\text{' mean' arterial pressure}}{\text{C O in litres/min}}$

Cardiac work (in arbitrary units) $=$ ' mean " arterial pressure $\times$ C O litres/min

TABLE III

Effects of 1 5 mg Digoxin intravenously

Case No	Reference No	Age and Sex	Diagnosis	Time	Rt auricular pressure cm saline above sternal angle	Heart rate	Cardiac output litres/min	Blood pressure mm Hg	% change in total peripheral resistance	% change in cardiac work
1	Q 11	M 64	Aortic stenosis S R	Before	— 2 5	100	3 1	115/75	—20	+26
				After	— 5 5	100	3 9	110/80		
2	Q 12	M 47	Aortic incompetence S R	Before	+18	106	2 3	135/90	—15 7	+34
				After	+ 6	106	2 9	148/90		
3	Q 13	M 61	Hypertension S R	Before	+ 7	92	2 6	170/130	— 6 4	+24 5
				After	0	88	3 0	190/135		
4	Q 14	M 43	Mitral stenosis Aortic stenosis S R	Before	+ 6 5	104	2 9	95/60	—30	+43
				After	— 1 5	98	4 15	95/60		
5	Q 15	F 33	Mitral stenosis S R	Before	+21	124	2 45	94/80	— 3 7	+60
				After	+11 5	120	3 15	126/90		
6	Q 16	M 62	Hypertension S R	Before	+ 8	105	3 05	200/130	—42	+75
				After	0	134†	5 3	200/134		
7	Q 17	F 45	Thyrotoxicosis Aur fib	Before	+32	190	3 04	120/80	—45	+81
				After	+17	130	5 55	120/80		
8	Q 20	F 54	Mitral stenosis Aur fib	Before	+ 6	160	2 5	95/	+ 5*	+84 5*
				After	— 3	115	3 3	130/-		
9	Q 21	F 44	Mitral stenosis Aortic incompetence Aur fib	Before	+ 7 5	155	2 3	120/80	—34	+81
				After	— 2 5	95	3 8	140/80		
10	Q 22	F 55	Mitral stenosis Hypertension Aur fib	Before	+17	130	2 8	190/90	—18	+35
				After	+11	86	3 6	210/85		
11	6 63	M 57	Hypertension S R	Before	+ 9 5	120	3 1	230/140	—31	+44
				After	— 2	108	4 46	230/140		
12	6 143	F 49	Mitral stenosis Aur fib	Before	+ 8	162	3 3	105/70	0	+24
				After	+ 4 5	146	3 65	118/78		

* Calculated from systolic blood pressure

† Desire to micturate

This method of obtaining the " mean " arterial pressure is open to criticism, but, within the range of pressure alterations observed, it probably gives a fairly accurate reflection of the changes in individual subjects

Results

Results are summarised in Table II and an example of a typical observation is shown in Fig 1 All cases showed a fall of right auricular pressure and, as might be expected, the larger the bleeding the greater the

fall All cases with initial right auricular pressure above the sternal angle showed a rise of cardiac output after venesection Two cases with initial right auricular pressure below the sternal angle level showed a fall in cardiac output and one showed no change Only two cases (No 12 and 13 with a falling output) showed significant increase in heart rate Venesection in cases with auricular fibrillation produced changes of cardiac output similar to those in patients with sinus rhythm
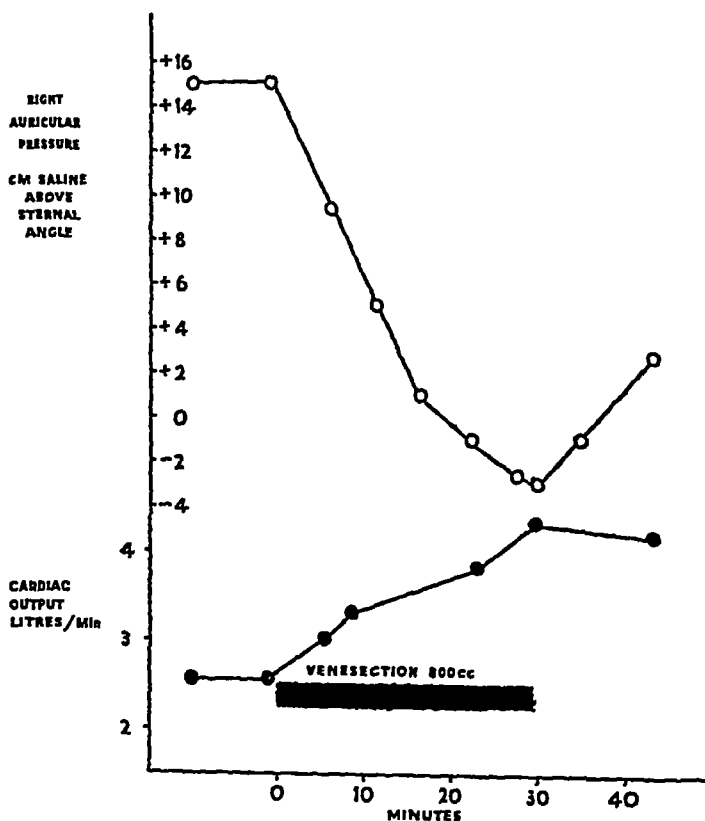
Fig 1 *Case 1* Hypertensive failure Bleeding causes a fall in right auricular pressure and a rise in cardiac output

All cases except two (one a case of complete heart block) showed some degree of blood pressure fall after venesection Since blood pressure ∝ cardiac output × total peripheral resistance, this indicates considerable diminution in total peripheral resistance which was reduced by 11 to 48 per cent (Table II) Calculation of cardiac work showed an increase in all congested cases Normal subjects and cardiac patients with initial auricular pressure below sternal angle level and falling cardiac output after venesection showed a decrease in cardiac work (Nos 11-13)
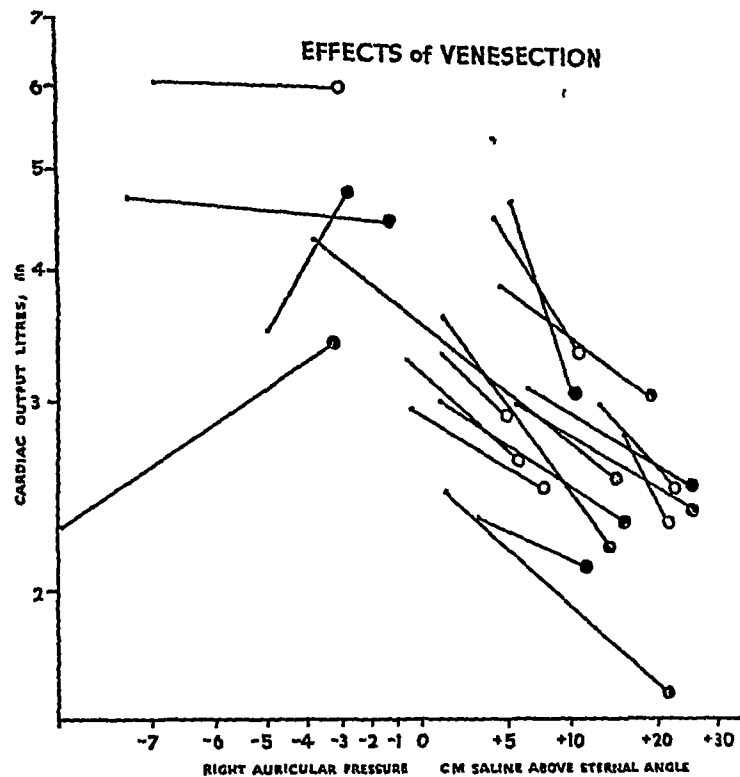
Fig 2   Effect of venesection (black circles) and cuffs (white circles) on right auricular pressure and cardiac output plotted on logarithmic scales   The logarithmic scale for right auricular pressure is arbitrary in Figs 2, 3 and 4 and zero represents the sternal angle   Dots and circles show initial data
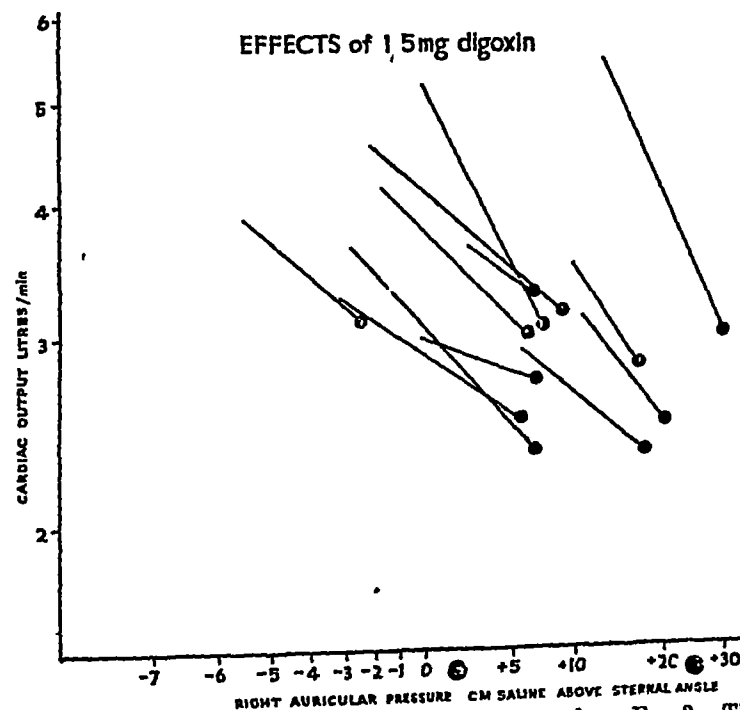
Fig 3   Effect of 1 5 mg of digoxin i v plotted on the same scale as Fig 2   The response to digitalis and venesection is similar

Comparison of digoxin and venesection effects

In a previous paper (7) it was shown that digitalis always caused a fall in right auricular pressure and that identical effects on right auricular pressure and cardiac output could be produced by trapping blood in the legs by congesting cuffs. This led us to suggest that the main effects of digitalis on cardiac output were caused by a primary action of the drug in reducing the venous filling pressure. In these experiments, however, the effects of digitalis and mechanical lowering of right auricular pressure could only be compared over a moderate range, as digitalis usually produced a much greater right auricular pressure fall than did cuffs on the thighs. These cuff experi-
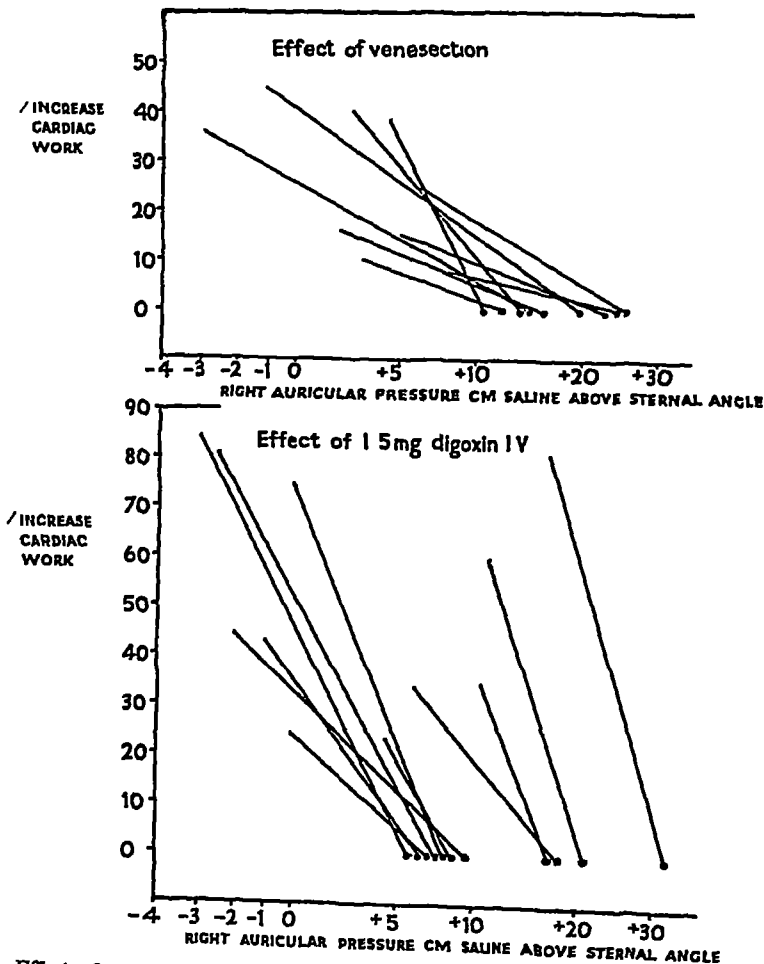
Fig 4 Effect of venesection and digoxin on cardiac work. There is a greater increase in cardiac work after digitalis than after venesection

ments were of short duration and were not accompanied by any significant change in arterial pressure

Further observations on digoxin action are added to our previously published data in Table III

A comparison of the effects of digitalis and venesection on cardiac output is shown in Figs 2 and 3, and there appears to be little difference between them   But while arterial pressure was lowered by large venesections (Table II), it was maintained or raised by digitalis (Table III), an effect already well known in animal experiments (2)   It is clear that the increase in cardiac work is less after venesection than after digitalis (Fig 4)
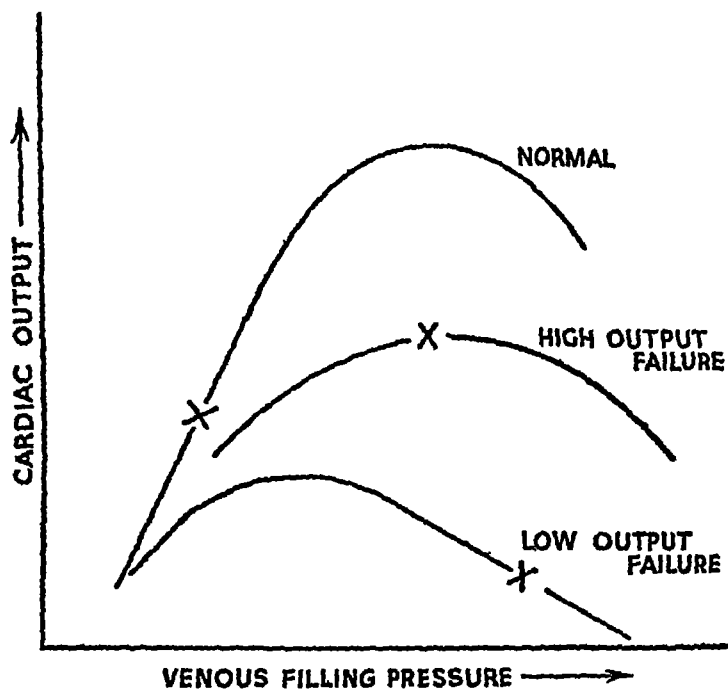
Fig 5   Suggested filling pressure cardiac output curves for normal subjects and cases of high and low output failure   Crosses represent usual status of each group at rest

Discussion

In general, the results confirm those obtained by trapping blood in the legs by congesting cuffs   Mechanical lowering of the filling pressure in the right auricle leads to increase in the output and work of hearts showing low output failure   As previously suggested, a curve relating cardiac output and filling pressure (8) affords a convenient concept for the interpretation of these results   The rise of cardiac output after venesection in cases of severe low output failure may be explained by considering such hearts to lie on the overloaded or falling part of Starling's curve where a further increase in filling pressure will cause a fall in output, or conversely, reduction of the filling pressure will cause a rise

This concept has been criticised by Dock (3), particularly because one case in the previous series (7) showed a slight rise in cardiac output, although the right auricular pressure was only slightly above the normal   In making the suggestion we were careful not to put any scale beside the curve, as we are aware that the curves are set at different levels in different cases of failure and that the level in the failing heart is different from that of the normal heart   We do not know how high the cardiac output can rise in a normal person, but it may certainly reach such figures as 25-30 litres per minute   The curves for low output failure, though of the same general shape, are clearly set at a different and much lower level of output   There are probably all gradations of curve from normal to the most severe, and in the final stages the curve may be nearly flat at the lowest level when no response to venesection or digoxin may be seen

The heart may be said to have failed when its response to venous pressure change lies on the overloaded part of Starling's curve, while the incipient stages of failure are present when the maximum possible output is conspicuously below the maximum attainable by the individual in health The influence of heart rate on the cardiac output-filling pressure curve is as yet ill-defined both in health and disease

It is unfortunately difficult to compare venesection and digitalis owing to the different responses of the blood pressure   After digitalis arterial pressure is higher than after venesection and the heart is doing more work It is possible that a closer comparison over a small range is afforded by the cuff experiments previously reported, where blood pressure changes are insignificant   To obtain a suitable control it would be necessary to raise arterial pressure after cardiac improvement following venesection to see if cardiac output would be maintained by the improved heart against increased peripheral resistance   The relation of cardiac size to cardiac work would also have to be compared after venesection and after digitalis

While the results seem to leave open the possibility of some direct stimulation of the failing heart by digitalis, reduction of venous pressure is clearly established as an important influence on the cardiac improvement which results from the use of the drug   It might be argued that the immediate action of venesection is of greater clinical benefit than digitalis since the output of the heart is increased with less expenditure of energy But the chief therapeutic difference appears to be in the long-term effects, for, while after venesection right auricular pressure tends to rise again, digitalisation often maintains the filling pressure of the heart at the lower and more beneficial level for an indefinite period

The reduction of total peripheral resistance after venesection suggests considerable arteriolar vasodilatation   The site of this vasodilatation is under investigation and will be the subject of a later report

SUMMARY

1   Cardiac output increases when right auricular pressure is lowered by venesection in cases of severe low output heart failure (valvular, hypertensive and ischæmic heart disease with initial auricular pressure above the sternal angle)

2   With this rising output there is usually a fall in blood pressure indicating considerable decrease in peripheral resistance   In spite of the fall in blood pressure, cardiac work is usually increased

3   Digitalis has a similar effect on right auricular pressure and cardiac output, but the blood pressure often increases   Cardiac work, therefore, shows a greater increase than after venesection   These results do not necessarily imply that digitalis has a direct stimulating action on the heart

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OBSERVATIONS ON THE HEADACHE ACCOMPANYING

FEVER

By RONALD BODLEY SCOTT and ROBERT P WARIN

THE past few years have seen a notable growth in our understanding of the mechanism underlying headache   This advance was initiated by Pickering's study of the headache which follows injection of histamine (9), and has been maintained by observations in migraine, arterial hypertension (5, 14, 15, 16, 17) and intracranial tumour (7)   The present paper is concerned with the cause of a common, but neglected, variety, the headache accompanying acute febrile disease

The Material

Observations were made on one hundred patients admitted to a Military Hospital in the Middle East, with headache and fever   The final diagnoses are given in Table I

TABLE I

Diagnoses of 100 patients with fever and headache

Sandfly fever	50
Malaria, benign tertian	28
Malaria, malignant tertian	1
Pneumonia, pneumococcal	2
Pneumonia, primary atypical '	2
Erysipelas	2
Typhoid fever	2
Dysentery, acute bacillary	1
Vaccination reaction	1
Short fever of uncertain origin	11
	100

So far as these diseases are concerned, this work has revealed no evidence that the mechanism of headache associated with fever differs with the infective agent   It is perhaps worth mentioning that none of the diseases here considered was associated with a meningitis or with changes in the composition of the cerebro-spinal fluid

Clinical description

At the onset of fever, headache was usually throbbing, increasing gradually to a constant severe pain exacerbated by each beat of the heart, and waning, with defervescence of the fever, to regain its throbbing character and finally vanish The pain was experienced in three areas bitemporal and frontal, spreading a variable distance towards the vertex (Field I), supra-nuchal occipital (Field II), and infra-nuchal occipito-cervical (Field III) These areas are illustrated in Figure 1 The sharp distinction all
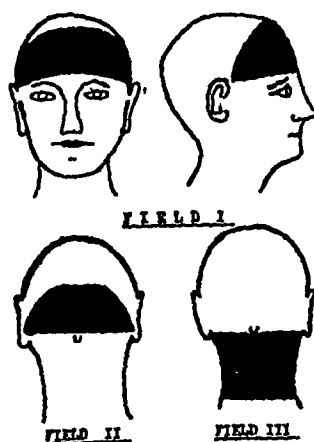
Fig 1 The areas to which headache was referred

patients made between Field II and Field III was striking Although headache was often experienced in Field I alone, pain in Fields II or III was always accompanied by pain in Field I, as headache faded it commonly left Field II and Field III before Field I In general the more severe headaches were referred widely, but to this statement there were exceptions No disease was accompanied by a headache of characteristic distribution, but pain in Fields I + II was common in sandfly fever Table II sets out the pattern of headache in these patients

TABLE II

Pattern of headache in 100 patients

	Sandfly fever	Malaria, benign tertian	Others	Total
Field I (alone)	30	15	12	57
Fields I + II	9	—	2	11
Fields I + III	10	11	6	27
Fields I + II + III	1	2	2	5

In 92 patients the headache was of equal intensity on the two sides , in the remaining eight, all with pain confined to Field I, it was either unilateral or notably more severe on one side   Four of these had previously had injuries either to the temple or to the eye of that side (mortar bomb wound with residual periosteal thickening ,   injury to right temple with pericranial hæmatoma ,   lime burn of right eye with corneal scar ,   fragment of steel removed from right eye) ,   one patient was the subject of hemicranial migraine ,   one had had a right frontal sinusitis ten weeks previously of which no sign remained when he was admitted with malaria ,   in two there was no cause to which the asymmetry of the pain could be ascribed

In 15 patients there was a history of recurrent headaches   In eight of these a psychic cause seemed probable, and in them there was no resemblance between the previous and the febrile headaches ,   four were prone to migraine and were unable to distinguish between the migrainous and the febrile headaches ,   one indeed, suffering from malaria, complained only of a severe attack of his customary migraine ,   one had had attacks of right temporal headache since a lime burn of his right eye eleven years previously, these were identical with his febrile headache ,   in two there had been vague headaches of uncertain nature

Pain referred to the eyes accompanied the headache in 93 patients   This pain was described as constant, boring, or aching ,   it was never throbbing ,   it usually appeared after the onset of headache and often remained for one or two days after the headache had vanished   The pain was said to be in or behind the eyes and was made worse by moving the eyes   There was tenderness on pressure on the globe in 87 patients   In 58 the pain and tenderness were equal in the two eyes ,   in 20 they were greater in the right eye, but in only four of these was the headache more severe on the right ,   in 15 they were greater in the left eye and two of these had left-sided headache   In two patients with right-sided headache pain and tenderness were equal in the two eyes   Because of the lack of correlation between the headache and the eye-pain, the two symptoms are considered separately in subsequent paragraphs

Rigidity of the posterior cervical muscles was present in only two patients ,   but pain on flexion of the neck was common in these muscles and in those of the back, in some it was associated with pain referred to the lower end of the sternum   When headache was experienced in Field I, flexion of the neck usually caused pain in the dorso-lumbar muscles, when in Field III, pain was more common in the neck   Kernig's sign was present in none

General relationships

The duration and severity of the headache and eyeache were related to the fever and to its accompanying circulatory changes   The close relationship between headache and fever is illustrated by Fig 2, the chart of a patient
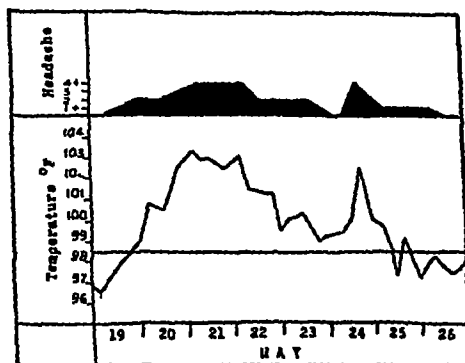
Fig 2   Headache in sandfly fever

with sandfly fever contracted while in hospital and by Fig 3   The correspondence was, however, not absolute for headache persisted sometimes for two or three days after fever had ceased, and was common in the early morning for the first few days of convalescence   The circulatory changes associated with fever and headache were as follows   peripheral vasodilatation was obvious, the temporal arteries were prominent and vigorously pulsatile, capillary pulsation in the skin and arterial pulsation in the retinæ could be seen, the face and eyes were suffused, the pulse quick, the pulse pressure raised and Duroziez' murmur often audible
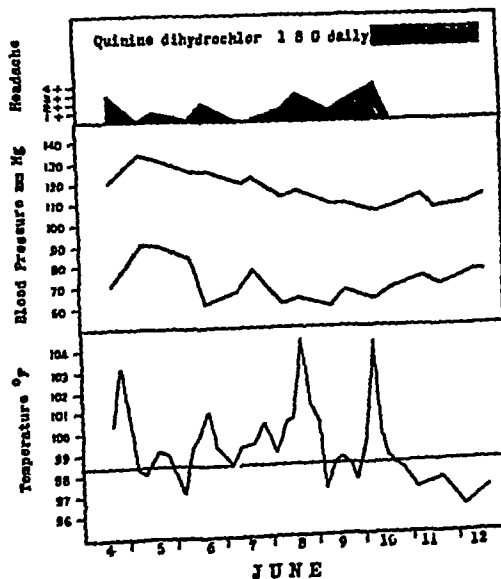
Fig 3   Relations of headache, fever and blood pressure in a case of benign tertian malaria

The arterial blood pressure showed no constant alteration   Repeated readings were made in 25 patients   the systolic figure commonly fell during four or five days, by 20-30 mm of mercury , the diastolic was more variable, lying usually between 50 and 60 mm of mercury   Figure 3 shows the blood

pressure readings in a man with benign tertian malaria and is representative of the changes seen in other patients   In this series there were no patients with arterial hypertension

The local mechanisms of the headache

Headache is almost always a referred pain , it depends anatomically on some structure where a local disturbance arouses pain-impulses, and nervous pathways by which the sensation is referred to the head   The nervous pathways concerned in headache probably lie in the fifth cranial and upper three cervical nerves, and less frequently in the ninth and tenth cranial nerves (12, 15)   The cranial structures which are sensitive to pain have been studied at operation by neurosurgeons (4, 7, 8, 11)   Apart from the cranium and its overlying structures, such sensibility is possessed only by the dural sinuses and their main tributaries, the arteries and adjacent parts of the dura, the large basal intracranial arteries and possibly the choroid plexus   Stimulation of these evokes a sensation of head-ache experienced in an area depending on the structure stimulated It is logical to suppose that the disturbance which causes headache in the sick patient is situated in one or more of these pain-sensitive structures

The nature of local disturbance   There is abundant evidence that the local disturbance which arouses pain-impulses depends, in many types of headache, upon a dilatation of intra- or extra-cranial arteries, leading to an increase of tension in the vessel wall or perivascular tissues   Such a mechanism exists in migraine (5, 14, 15, 16, 17), in the headache following intravenous injection of histamine (1, 2, 9), and in that of hypertension (14) Observations on febrile headache have been few, but Pickering (10), in one case, found it similar to that induced by histamine   Sutherland and Wolff (14), in patients made febrile by intravenous injection of typhoid vaccine, showed the headache to parellel the increase in amplitude of the temporal pulse and of the oscillations in the cerebrospinal fluid pressure which occur synchronously with the heart beat   These authors also described dilatation of arteries, seen through a window in an animal's skull, after intravenous injection of foreign protein   They concluded that headache in fever resulted from dilatation of intracranial arteries

The arterial origin of febrile headache was borne out by observations in the present series   The circulatory changes, already described, provided evidence of arterial dilatation   Obliteration of one common carotid artery by pressure with the finger relieved the pain on that side of the head and, when the vessel was released, headache returned with an intensity which frequently caused the patient to cry out   Additional support was given by the effect on the headache of changes in the arterial blood pressure   the pain was made worse by sudden straining, an act which Pickering (9) has shown to raise the pressure by some 25 mm of mercury   In three subjects, at the end of the fever when headache was minimal, 0 8 to 1 0 mg of

adrenalin hydrochloride was injected intramuscularly, with the rise in arterial pressure the intensity of the headache increased, to wane as the pressure fell (Fig 4) This procedure caused no headache in normal persons Conversely, a fall in arterial pressure was accompanied by relief of headache In four subjects inhalation of amyl nitrite was followed by disappearance of the pain which returned to its previous intensity as the arterial pressure rose again to normal (Fig 5) One patient received an intravenous
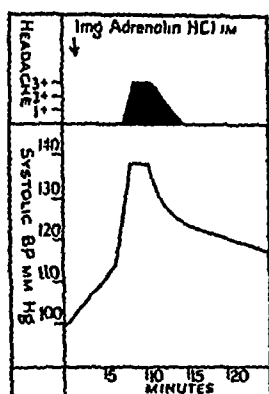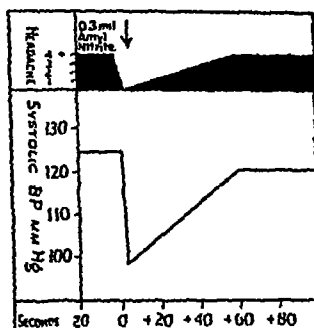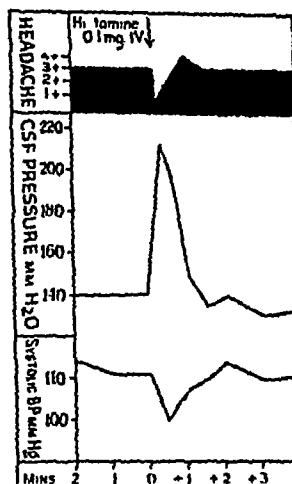
Fig 4   Fig 5   Fig 6

Fig 4   Effect of adrenalin hydrochloride 1 mg intramuscularly in a male aged 24 years with sandfly fever on the first day on which the temperature was normal Headache was not present at rest but was produced by straining or head shaking   T 98 2, P 60

Fig 5   Effect of amyl nitrite—in a male aged 30 years with sandfly fever   T 102 6°F, P 88

Fig 6   Effect of intravenous injection of histamine acid phosphate in a man aged 24 years with benign tertian malaria and severe headache   T 103 8°F, P 96

injection of 0 10 mg of histamine acid phosphate while the headache was at its height, and the subsequent changes in blood and cerebro-spinal fluid pressure were recorded The sudden fall in arterial pressure and coincident rise in that of the cerebro-spinal fluid, described by Pickering (9), were noted and, during this phase, the headache lessened, when the two pressures had returned to their former levels the intensity of the headache was accentuated for a few seconds, to revert rapidly to that existing before the injection (Fig 6)

These experiments showed that the headache was made worse when the force distending the cranial arteries was increased by a rise of blood pressure, conversely, that a fall in blood pressure was attended by a lessening of the headache They provide further evidence that the disturbance which gave rise to headache was an increase in tension in the walls of the cranial arteries

The site of the local disturbance The region in which a headache is experienced gives no exact indication of where the exciting stimuli are arising, it has been shown, for example, that a pain felt in the temple may be provoked by stimulation of either the temporal artery or various intracranial structures (11) Thus the seat of the disturbance must be inferred from other evidence

Wolff (15, 16) claims that the headaches of migraine and arterial hypertension are due to stimuli arising in dilated extracranial arteries but that the disturbance is intracranial in histamine headache Pickering (10) and Sutherland and Wolff (14) regard the headache of fever as comparable with that caused by histamine, our observations were not in complete accord with this suggestion They can best be explained by recognising three varieties of febrile headache in the first the exciting impulses arise within the skull, in the second from extracranial arteries, and in the third from both these sources The evidence for this hypothesis is set out in the following paragraphs

In 52 patients pressure sufficient to obliterate one temporal artery caused no lessening of the headache on that side, although there was complete ipsilateral relief on obliteration of one common carotid artery This was taken to indicate that the stimuli producing headache were not arising, to any significant degree, from the temporal artery but were of intracranial origin The headache in 29 of these patients was located in Field I, in five in Fields I + II, in 13 in Fields I + III, and in five in Fields I + II + III In all patients the existing headache was made worse by shaking the head, a phenomenon denoting an intracranial origin, for movement of the head cannot alter the stresses on the fixed vessels of the scalp or calvarium

Ten of these patients were subjected to lumbar puncture, the cerebro-spinal fluid pressure was significantly raised in none, lying between 80 and 180 mm of fluid There was thus no relation between headache and rise in cerebro-spinal fluid pressure, indeed it has been shown by Northfield (7), that increase in intracranial tension does not, of itself, cause headache The fluid was chemically and cytologically normal in all cases The pressure changes synchronous with the heart beat were unusually great Jugular compression led to a rise in pressure to 220-350 mm and, with this rise, there was relief of headache When the jugular veins were compressed after removal of 12-15 ml of cerebro-spinal fluid there was no rise in pressure and the headache was made worse

These observations are identical with those of Pickering (9) in histamine headache and he has offered the following explanation Jugular compression has two mutually antagonistic effects first, the sudden distension of the intracranial venous channels leads to a rise in intracranial pressure which decreases the difference, within the skull, between the intra- and extra-arterial pressures, and thus reduces the effective force distending the arteries,

secondly, the increase in pressure within the veins is transmitted to the arteries, increasing the distending force With jugular compression, before thecal drainage, the first effect overshadows the second , but, after drainage, distension of the venous sinuses is no longer sufficient to raise the intracranial pressure, and the second effect predominates In the first instance, therefore, headache is relieved and, in the second, increased

The effects of intravenous injection of histamine, cited above, are relevant to this discussion In the normal subject this drug is followed by severe headache lasting some twenty minutes which has been shown to depend on dilatation of intracranial arteries (1, 2, 9) Intravenous injection of histamine in the febrile patient caused an increase in headache only for a few seconds, suggesting that the mechanism usually productive of histamine headache was already in operation

It is submitted that these observations furnish evidence that febrile headache of this type was analagous to histamine headache and was the consequence of an increase in tension in the walls of dilated intracranial arteries

In 15 patients with headache experienced in Field I, pain was abolished on one side by compression of the ipsilateral temporal artery All eight patients with unilateral headache fell into this group , in them there was always a visibly and palpably fuller temporal pulse on the affected side In five patients with pain referred to Fields I + II, pain in Field I was relieved by temporal compression, and in Field II by compression of the occipital artery Ray and Wolff (11) have shown that stimulation of this vessel gives rise to pain localised in an area corresponding with Field II

Jugular compression in this group always made the headache worse Lumbar puncture was done in two patients , the cerebro-spinal fluid pressures were 170 and 200 mm of fluid , jugular compression raised the pressure to 300 mm and 400 mm and was accompanied by increase in headache Reduction of pressure to below 100 mm , by drainage of fluid, did not affect the headache These observations can be explained by the rise in extra-cranial venous pressure being communicated to the extracranial arteries and increasing the tension in their walls , the cushioning effect of the coincident rise in intracranial pressure would not, of course, be exerted on vessels outside the skull

Head-shaking had an inconstant effect, producing no aggravation in any, but in some a pain in the region of the nasion , suggesting that it had no action on the pre-existing headache but might be sufficient to excite pain from intracranial structures

This second type of headache, it is submitted, was excited by stimuli arising in dilated extracranial arteries, particularly the temporal and occipital vessels It was comparable to the headaches of migraine and arterial hypertension (14, 15, 16) In three patients the headache was not relieved by intravenous injection of 0 25 mg of ergotamine tartrate

The remaining 28 cases fell into neither of the foregoing groups   in them temporal compression sometimes relieved the pain in Field I, and sometimes diminished it   This could be shown by simultaneous compression of the common carotid and temporal arteries of one side   release of the first would be followed by some return of pain, and subsequent release of the second by a further increment   Jugular compression gave equivocal results, sometimes diminishing, sometimes not affecting, the pain   When, however, the temporal artery of one side was obliterated, jugular compression was accompanied by relief of headache everywhere but in the contralateral temple

These patients presented features common to both of the previous groups and it is suggested that in them painful stimuli were arising from dilated arteries both inside and outside the skull

The eye ache

For reasons previously stated, eye ache is considered separately from headache   It was clear that the pain in the eyes was related to the vascular disturbance, for it was diminished or relieved in one eye by obliteration of the carotid pulse of the same side   Such pain might have its origin in a local disturbance within the orbit, or might be referred from other intra- or extra-cranial structures   The improbability of its intracranial origin was suggested by its frequent persistence after headache had vanished, and by the observation that jugular compression always increased the pain in the eyes even when it relieved headache   Compression of the temporal artery did not affect the eye pain, nor has pain in the eye been described with experimental stimulation of this vessel   It seemed probable, therefore, that it had a local origin within the orbit, and this contention was supported by the frequent inequality of the pain in the two eyes, by the tenderness and by the pain on movement of the globe

Eckardt, McLean and Goodell (3) have shown that ocular pain may be caused by stimulation of the conjunctiva, by an increase in ocular tension, and by traction on the iris or on the extrinsic eye muscles   No information is available concerning the sensitivity of the orbital vessels

Although the eye ache appeared fundamentally dependent on the vascular changes, it seemed unlikely that the mechanism was identical with that of the headache   If pain-impulses had been arising from an increase of tension in the walls of dilated orbital arteries, pressure on the globe, tending to equalise the intra- and extra-arterial pressures within the orbit, should have decreased the pain , in fact, such pressure commonly had the reverse effect

In many patients, palpation suggested that the ocular tension differed in the two eyes, and that pain was greater in the eye of higher tension   Repeated readings were made with a Schiøtz tonometer in 18 patients and this asymmetry confirmed   The highest reading was 40 mm of mercury and in

only five did it exceed 30 mm   There was little correlation between the tension and the eye ache, although pain was severe in all patients with readings above 30 mm   There was often a fall of 5-10 mm with the disappearance of eye ache but it is improbable that pain could have been due to so small an increase in tension

In 16 patients eserine was instilled into one or both eyes   In four there was complete relief of pain, in five partial relief and in seven no effect headache was unaltered   With relief of pain, a previously raised tension fell , in one case, from 31 5 mm of mercury to 15 mm in the right eye, and from 28 mm to 19 mm in the left   A fall in tension was not necessarily accompanied by relief of pain

In 11 patients, homatropine sulphate was instilled into one eye and normal saline into the other   in five the pain and tenderness were unaffected, in six they were made worse in the eye receiving homatropine for a period of a few hours   There was commonly a rise in tension of 4-6 mm of mercury

In three patients, adrenaline hydrochloride was instilled into one eye with reduction of conjunctival suffusion and slight decrease in pain on that side

The deductions to be drawn from these observations were not obvious It was probable that the fundamental cause of the eye ache was the vascular disturbance within the orbit   There was no constant or significant increase in ocular tension   The effects of eserine and homatropine were equivocal It seemed likely that the mechanism of the eye ache was more complex than that of the headache, and, although a symptom of the same circulatory changes, was not the immediate result of an increase in tension in the arterial walls

Discussion

The characteristics of the headaches experienced by these febrile patients have been described, and the mechanism of their production discussed, in previous paragraphs   Evidence has been adduced that the stimuli evoking the sensation of headache were generated in dilated cranial arteries   in some, the arteries concerned were within the cranium , in some, they were extracranial , and in the remainder, impulses producing pain arose in arteries both inside and outside the skull   Associated with the headache there was usually pain in the eyes which appeared to arise from a complex vascular disturbance within the orbit   Pain in the cervical and dorso-lumbar muscles on flexion of the neck was of common occurrence   This symptom is, in part, explained by the studies of Simons, Day, Goodell and Wolff (13) who recorded the electrical changes in the cervical muscles, and showed that in spontaneous and induced headaches there was sustained muscular contraction which was at times sufficient to cause local pain

Certain points, irrelevant to the main theme of this paper, are of sufficient interest for comment. A high proportion of the patients with

pain more intense in one temple had a previous history of injury within the area innervated by the trigeminal nerve of that side , this connection has been observed by Horton (6) in a patient who, following ulcerative keratitis, experienced attacks of " right reflex hemicrania " which were relieved by ergotamine tartrate It is possible that, through disease or injury, the arteries of an area may become more susceptible to vasodilator agents Secondly, in the migrainous, the headache provoked by fever was indistinguishable from that of the migrainous attack, suggesting that the arteries whose dilatation occasions the headache of migraine show a preferential response to the stimulus of fever Finally, the early morning, a common hour of onset for the headaches of migraine, arterial hypertension and cerebral tumour, was often marked by an exacerbation of febrile headache , it is, perhaps, a time at which vasomotor tone is unusually labile

SUMMARY

1 The clinical characteristics and mechanism of headache accompanying acute febrile illnesses have been studied in 100 patients admitted to a military hospital in the Middle East

2 Evidence is presented indicating that in febrile headache pain arose from distension of the walls or perivascular tissues of dilated cranial arteries In one group of cases, the pain impulses arose from intracranial arteries, in another from extracranial arteries, and in a third from both sources

3 Eyeache which accompanied headache in 93 patients, was probably due to an orbital vascular disturbance, the precise nature of which has not been determined

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OBSERVATIONS ON THE MECHANISM OF PAIN IN ULCER OF THE STOMACH AND DUODENUM

PART I—THE NATURE OF THE STIMULUS

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THERE is still much difference of opinion as to the source of pain and the nature of the pain producing stimulus in disease of the abdominal viscera The confusion has arisen partly from the relative inaccessibility of the viscera but chiefly from a general reluctance to put hypothesis to the test of measurement and experiment Because of the frequency of ulcer of the stomach and duodenum, because of the striking and relatively constant characteristics of the pain which constitutes their chief symptom, and because the viscera concerned are more accessible than most to the physician, these maladies have been selected for study

The factors influencing its onset and subsidence suggest that the pain of peptic ulcer is related to the state of the gastric content Thus pain tends to follow a meal after an interval which varies with the position of the ulcer, the interval being of the order of an hour in ulcers near the incisura of the stomach and three hours or more in duodenal ulcers The ingestion of food always relieves and usually abolishes the pain of duodenal ulcer Food often relieves the pain of gastric ulcer, but frequently in this condition the patient has never tested the effect of food because the pain has disappeared or has been otherwise abolished before the next meal is due , or because the patient has been too nauseated to eat while pain was present Vomiting relieves the pain nearly always in gastric and sometimes in duodenal ulcer, though in the latter, vomiting is not common Finally, in all these varieties of peptic ulcer, pain is relieved by alkali

Concerning these facts, established by clinical observation, there is no dispute They are most simply interpreted as signifying that pain is due to

the attainment of an adequate degree of acidity in the gastric content, for
the three procedures which relieve pain, ingestion of food, ingestion of alkali
and vomiting have but one common factor, namely the removal from the
stomach of hydrogen ions   This hypothesis has received powerful support
from the work of W L Palmer (13, 14, 15, 16, 17), who showed in 1926 that
the injection of 200 c c 0 5% hydrochloric acid into the stomach would
reproduce the pain in most patients with gastric, duodenal and anastomotic
ulcer when they were experiencing spontaneous pain, but not usually in the
same patients during the period in which, as a result of treatment, pain was
absent   He showed that the pain of ulcer can be relieved by aspirating,
and reproduced by reinjecting, the gastric content through a tube lying in the
stomach , and he found close agreement between the titratable acidity of the
gastric content removed during spontaneous pain and that during pain
produced by injection of acid   He concluded that hydrochloric acid was the
irritant common to all the solutions which constituted an adequate stimulus
to the pain producing mechanism, and that since pain could also be produced
by sulphuric and acetic acids and by caustic soda, HCl probably acted as a
chemical irritant   Finding that HCl would also produce pain in gastric
carcinoma but not in conditions unassociated with an organic lesion of the
mucous membrane he believed the pain mechanism only to be sensitive in the
presence of an organic lesion of the mucous membrane of the stomach or
duodenum

Nevertheless the hypothesis has not been generally accepted, particularly
in this country, largely owing to the views of Hurst   During his observations
on the sensibility of the alimentary canal in 1911 Hurst (9) concluded that
the stomach was insensitive to acid, for the introduction into the stomach
of 4 oz (114 c c) 0 5% HCl produced no sensation in normal subjects nor
in six patients with gastric ulcer   Finding that rapid inflation of a balloon
in the stomach did give rise to pain he supposed that tension was the only
stimulus to which the pain receptors in the stomach were sensitive, that pain
in peptic ulcer was due to tension, and that the part played by acid was
subsidiary and due to reflexes from the ulcer closing the pylorus and
stimulating peristalsis   In 1926 Ryle (20) went further than Hurst in rejecting
completely the role of acid, because he had seen many cases of ulcer with
extreme hypochlorhydria or even achlorhydria in which pain was as severe
as in cases with normal or high acidity   In 1929 Hardy (8) repeated Palmer's
observations and though he confirmed in the main the effects of acid
injection in active and quiescent ulcer, he found that acid would produce
pain in other conditions and, finding from a detailed consideration of his
results many discrepancies, rejected the hypothesis that acid was the normal
stimulus to pain in peptic ulcer   Christensen (6) in 1931 also found no
relationship between the titratable acidity of the gastric juice (to Congo
Red, Phenolphthalein and Gunzberg's reagent) and the occurrence of hunger
pain

In developing the hypothesis that pain in ulcer arises through tension in the wall of the stomach Hurst (9) originally supposed pain in both gastric and duodenal ulcer to be due to distension of the pyloric vestibule through increased gastric peristalsis working against a closed pylorus, and attributed the different times of onset of pain to the different times at which stimulation of the ulcer by acid reflexly exaggerated peristalsis and pyloric contraction Later (10) he modified his views, regarding distension of the pyloric vestibule as the cause of pain in duodenal ulcer and distension of the cardiac end of the stomach as the cause of pain in gastric ulcer, the stomach in each case being divided by contraction of its wall into two cavities during the occurrence of pain Ryle (20) also accepted contraction of the stomach as the cause of pain but differed from Hurst in supposing that the delayed pain of duodenal ulcer was due to an increase in tone of the stomach as a whole, and attributed relief by food and alkali to the decrease in tone following increased gastric content, in the case of alkali through liberation of CO_2, the immediate pain of gastric ulcer he supposed to be due to localised increase of tension in the wall of the stomach opposite the ulcer , here relief by alkali was attributed to belching and diminution in tension in the proximal part of the stomach * In 1928 Bolton (2) and Poulton (18) both supported the tension hypothesis of pain None of these writers produced any direct evidence in favour of their hypothesis, and their chief support lay in superficial similarities between the hunger pain of duodenal ulcer and the hunger pangs of normal subjects which Cannon and Washburn (4) and Carlson demonstrated to be associated with large contraction waves occurring in the nearly empty stomach However, some evidence has been produced that pain in peptic ulcer may be associated with gastric contractions The most impressive records are those of Carlson (5) and Christensen (6) Carlson investigated one case by means of a balloon in the fundus and body of the stomach and recorded pains of moderate severity synchronous with contractions of the stomach which were not greater than those felt as hunger pangs in the days when the patient had no pain Christensen investigated 18 patients with hunger pains, irrespective of their diagnosis, by means of a balloon in the cardiac end of the stomach and found that pain was never present without contractions of the stomach, but in the vast majority of patients the contractions were of the normal amplitude and frequency "It may even happen in the same gastrographic examination that one contraction period is associated with pain, whereas the patient is feeling perfectly well during another contraction period which does not differ from the first one in duration or intensity " She accepted contraction of the stomach as being one factor in the production of the pain because in some patients with intermittent pain, contractions and pain were simultaneous , but clearly another factor was involved and, not finding the titrable acidity

* Ryle believed that the alkalis commonly used in therapeutics acted through the release of CO_2 It is now agreed that magnesium trisilicate and aluminium hydroxide have a qualitatively similar effect on pain

related to pain, she supposed that this second factor was the varying state of inflammation of the gastric and duodenal mucosa It may be noted that the only positive evidence for gastric contractions being the cause of ulcer pain in these two papers was the occurrence of spasms of pain synchronous with gastric peristalsis It can be stated that this pain is not the usual pain of peptic ulcer which is a steady ache characteristically lasting several minutes or hours On the other hand Ortmeyer (12) and Palmer (15) have failed to obtain evidence that gastric contraction is a common factor in ulcer pain Ortmeyer recorded from a balloon in the pyloric end of the stomach in 29 patients with gastric and duodenal ulcer and found no relationship between naturally occurring pain relieved by alkali and either peristalsis or tone Palmer, recording from a balloon in the stomach or duodenum and producing pain by intragastric injection of HCl, observed pain without contraction 108 times and intermittent pains synchronous with contractions nine times

The tension hypothesis has also been explored by viewing the stomach and duodenum by X-rays Reynolds and McClure (19) gave a meal of ground beef and barium sulphate and examined the stomach and duodenum at suitable intervals They found that the occurrence of pain in patients with gastric and duodenal ulcer was not related to gastric peristalsis, localised spasm of the stomach or spasm of the pylorus Palmer (15), using a barium sulphate emulsion containing 0 5% HCl to produce pain, observed in patients with gastric or duodenal ulcer no phenomena in the presence of pain which were not observed in its absence , in particular no relationship was observed between the presence or absence of pain and gastric peristalsis or the condition of the duodenal cap , in duodenal ulcer pain did not occur until the acid emulsion had passed the pylorus Wilson (21), examining duodenal ulcer patients during pain with ordinary barium emulsion, *found no evidence that gastric peristalsis or pyloro-spasm was the cause of pain*, but finding that in 13 cases filling of the duodenal cap by manual pressure on the abdomen was followed almost immediately by relief of pain he suggested that pain might be due to contraction of the duodenal caput The character of the pain and the tension necessary to produce it when a balloon is inflated in the stomach do not offer impressive support to the hypothesis Thus Bloomfield and Pollard (1) found that the " main feature of the referred sensation from gastric inflation was its indefinite quality," and that a pressure of 40 mm Hg was necessary to produce it

In view of the conflict of evidence and of differences in its interpretation, it seemed desirable to investigate the problem afresh In this paper it is proposed to consider the nature of the stimulus to pain, and in the next, the site of the afferent nerve endings on which it acts In this paper then we shall consider first the relationship between gastric acidity and pain, and second the relationship between gastric contraction and pain

The observations have been made on 55 cases of peptic ulcer located in the stomach in 27, the duodenum in 20, both stomach and duodenum in 2,

the pylorus in 2, and the surgical anastomosis between stomach and jejunum in 4   The diagnosis in these cases has been made by clinical and radiological examination, and confirmed by ·gastroscopy in 4, by operation in 9, and by autopsy in 1   In addition, observations were made on three cases of carcinoma of the stomach verified by operation   The patients have been selected from the material available to us on two grounds, firstly because they had naturally occurring pain during their stay in hospital and secondly because they were intelligent and co-operative   Throughout these observations we have been careful to avoid suggestion, merely instructing the patient to record the time of onset and disappearance of pain and to indicate to us, as accurately as he could, its severity

THE RELATIONSHIP BETWEEN GASTRIC ACIDITY AND PAIN

The observations here described have been made to determine first whether the onset and subsidence of naturally occurring pain are related to changes in introgastric acidity, second whether artificially induced acidity of the gastric content will reproduce the same pain, and third whether the degrees of gastric acidity associated with naturally occurring and artificially induced pain correspond   Before describing these observations it is, however, desirable that we should refer briefly to two possible sources of error in the methods used to study gastric acidity

Methods and their sources of error

1   *Hydrogen ion concentration*   The conventional method of determining the acidity of gastric contents is by titrating the filtered sample with $\frac{N}{10}$ NaOH using Töpfer's reagent and phenolphthalein as indicators   The gastric content always contains buffers particularly when undigested or partly digested food is present   The " free and total acid " thus represent two points on the titration curve of a buffered solution , and while Michaelis (11) has found that the HCl content determined by titration to the point at which Töpfer's reagent becomes salmon red usually agrees with the hydrogen ion concentration determined electrometrically there is no guarantee that this is always so   Further the end-points in the titration are by no means sharp, and variations in lighting and in estimation of colour, for colour standards are rarely used, are usually uncontrolled   For these reasons we have measured the hydrogen ion concentration electrometrically with the double quinhydrone electrode on the unfiltered gastric samples

2   *Sampling the gastric contents*   The hydrogen ion concentration of the gastric contents is determined by the hydrochloric acid secreted by oxyntic cells which are confined to the body of the stomach, by dilution with other gastric secretions and saliva, by ingested food and its breakdown products acting as buffers, by the secretion of alkaline mucus throughout the stomach and by reflux of the alkaline duodenal contents through the

pylorus   Even though the waves of peristalsis tend to mix the fluid contents, it is possible that the acidity may differ in different parts of the stomach That this in fact happens is shown by Fig 1, one of two similar curves from a patient with duodenal ulcer in which the hydrogen ion concentration of the gastric fluid was measured on samples obtained simultaneously from two Ryle's tubes, one in the pyloric antrum, the other in the body of the stomach, the position of the tubes being verified by X-rays, As might have been
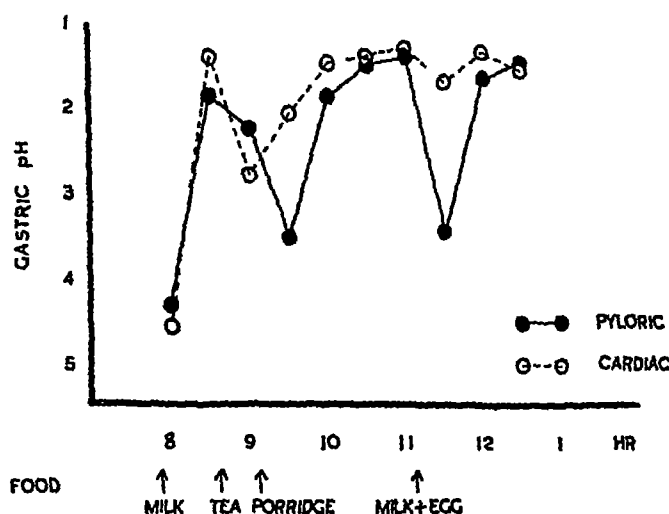
Fig 1   Subject C A (duodenal ulcer) 30 1 45   Shows the pH of samples of the gastric contents obtained as near as possible simultaneously from the pyloric and cardiac ends of the stomach Two Ryles tubes were swallowed before the observation and the position of their tips in the pyloric antrum and cardiac end of the stomach verified under the X ray screen

anticipated on theoretical grounds, the pyloric samples become much more alkaline than the cardiac, just after food is ingested   As digestion proceeds, both samples become more acid, the pyloric more rapidly, so that the acidities tend to become identical

It seems clear, therefore, that even if it is the acidity of the gastric content which determines pain in gastric and duodenal ulcer, it is to be expected that there will be some discrepancy between the occurrence of pain and the acidity of samples of the gastric juice   We may also infer that this discrepancy will be less obvious as the interval after the last meal increases

The effect on pain of withdrawing and reinjecting the stomach contents

(a) *Gastric ulcer*   The following protocol is illustrative

1 R C F   Male *aet* 37   Acute gastric ulcer

14 5 42	3 0 p m	Tea (boiled egg, bread, weak tea)
	3 25	pain begins
	5 50	pain severe
	5 55	Ryle's tube passed
	6 0	pain as before, aspiration begun
	6 6	132 c c out, pain less

6 10   210 c c out pH 1 58, pain very slight
6 10 6 14   stomach washed out, pain very slight
6 38 6 40   no change   100 c c original contents reinjected
6 41   no change
6 42   pain worse
6 50   pain bad   Aspiration begun
6 57   167 c c out pH 1 66 pain better, now very slight
7 0 7 3   stomach contents made alkaline with pulv alk and reinjected
7 4   pain gone
7 15   pain still absent   Sample of gastric content pH 7 37

In this observation severe pain was relieved but not abolished by withdrawing the stomach contents, aggravated by reinjecting them, relieved by again withdrawing them and finally abolished by reinjection after neutralisation It seems clear that the factor in the gastric juice producing pain was the hydrogen ion, since the other constitutents and the volume were unaltered in the last injection which actually abolished pain

Altogether in 12 observations on 8 cases of gastric ulcer, emptying the stomach through a Ryle's tube relieved or abolished the pain during the aspiration of 30 to 300 c c of liquid in 1 to 10 minutes , the pH of the fluid removed varied from 1 53 to 4 5 Reinjection of the fluid removed reproduced the pain in 1 to 10 minutes in 7 out of 8 observations on 5 cases , and second aspiration relieved the pain in these 7 instances In one case, in addition to that quoted, reinjection of the neutralised gastric contents failed to produce the pain

(b) Duodenal ulcer

Protocol 2   W.J D   Male aged 55   Large chronic duodenal ulcer floored by pancreas
6 7 43   3 0   p.m   pain severe since midnight not eased by milk   Ryle's tube in
       3 25   pain same   Aspiration begun
       3 46   pain which has been lessening during aspiration has now gone
       3 48   470 c c out (pH 1 39)
       3 49 3 58   contents returned to stomach
       4 5   pain has returned
       4 20   pain bad   aspiration begun
       4 26   470 c c out (pH 1 91) Pain gone   8 g (approx) sodium bicarbonate added to stomach contents (pH = 4 63)
       4 32 4 37   stomach contents so treated returned
       4 50   still comfortable
       5 0   slight discomfort
       5 32   discomfort definitely present   Aspiration begun   120 c c out (pH 1 68)   Discomfort gone

Here again we see that emptying the stomach abolishes pain which is reinduced by reinjection, relieved by aspiration, and not reproduced by injecting the stomach contents after reducing their acidity In this instance there was a slight return of pain 28 minutes after injecting the partly neutralised stomach contents, but this pain was relieved by aspiration and the stomach contents were again found to have become highly acid Altogether pain was abolished in 6 observations on 6 cases of duodenal ulcer by withdrawing 100 to 470 c c gastric contents in 3 to 18 minutes, the acidity varying between pH 1 28 and 1 73 In 4 of the observations the aspirated

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* A mixture of equal parts of calcium sodium and magnesium carbonates

fluid was reinjected, pain being reproduced in 3 in 2½ to 6 minutes, in 2 of these 3 instances the stomach was again emptied with relief of pain

(c) *Gastric cancer* In one case, J S, male, aged 55, with a large ulcerating cancer of the fundus, pain was relieved by aspirating in 5 minutes 100 c c of blood stained contents (pH 1 2), the pain disappeared 11 minutes later Replacing the fluid in the stomach reinduced pain in 3 minutes, pain becoming severe in 16 minutes, when the pH of the gastric contents remained unchanged 200 c c milk and raw egg were now introduced with considerable increase in pain (pH of gastric content 5), 16 minutes later when pain was still severe, 1 drachm of alkaline powder in 60 c c water was introduced Four minutes later (pH of gastric content 7) the pain started to ease and gradually disappeared over a further 28 minutes In this case it seems clear that at least two factors were involved in producing the pain, the hydrogen ion concentration and the volume of the gastric contents, and it is interesting that this patient stated in his history that his pain was abolished by alkali always, by food occasionally

The relationship of the hydrogen ion concentration of the gastric contents to naturally occurring pain

If the acidity of the gastric contents is the determining factor in producing pain, then there should be a relationship between the fluctuations in the pH of the gastric contents and the onset and subsidence of pain In 18 cases samples of gastric contents were obtained through an indwelling Ryle's tube at 15 or 30 minute intervals over periods of 2 to 12 hours, during a part of which time the patient experienced pain, the patient in each case kept a record of the time of withdrawal of the various specimens and of his sensations

(a) *Gastric ulcer* In 6 cases records were obtained over 2 to 3 hours following the ingestion of 500 c c meat extract (Bovril), in 3 cases over 3 to 12 hours during which the patient swallowed his ordinary meals, the tube being removed just before and swallowed again immediately after eating In each of the curves so obtained pain occurred with the highest acidities recorded, and was absent with the lowest The highest acidity occurred in P H, aged 51, a male with a chronic gastric ulcer in whom pain began at pH 1 2 and was absent at acidities less than pH 1 6 At the other extreme was E M, a male, aged 43, with a gastric ulcer recurrent after gastroenterostomy, in whom pain occurred with acidity greater than pH 2 6 and was absent with acidities less than pH 3 0 An example which includes two periods of pain is shown in Fig 2 In this patient, with a large ulcer on the posterior surface of the lesser curve with floor formed by pancreas and liver as operation revealed, pain began when the gastric content became as acid as pH 1 5, a value not previously reached Pain began to ease as the acidity spontaneously declined to pH 1 65, and disappeared after the ingestion of

milk which reduced the acidity to pH 3 8 No pain was experienced during the next 3 hours during which the acidity varied, but was on the whole low The second period of pain began at pH 2 4 and increased as the acidity
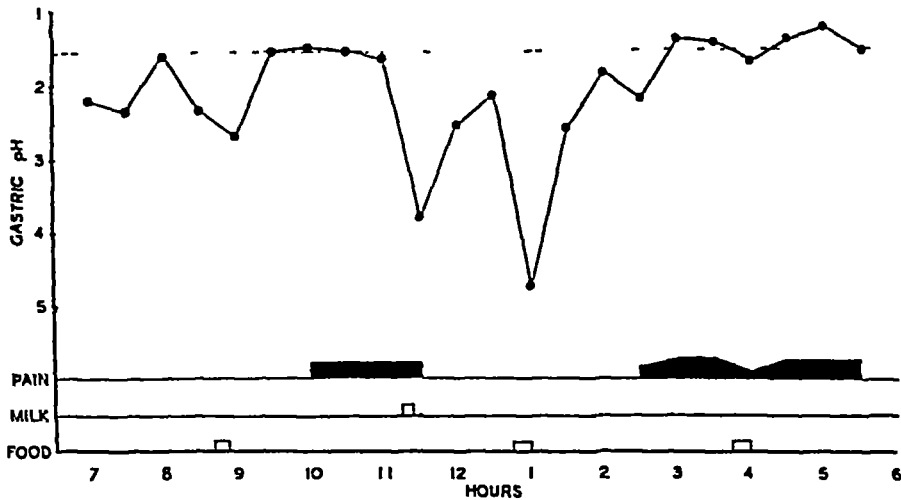
Fig 2 Subject C H with a large gastric ulcer measuring 3 5 × 3 0 cm on the X ray film and shown at operation to have eroded the pancreas, 17 2 45 Shows the pH of gastric samples removed from an indwelling Ryle s tube at half hourly intervals The patient kept a record of the times at which he removed the samples and of his pain The dotted line represents a pH of 1 55
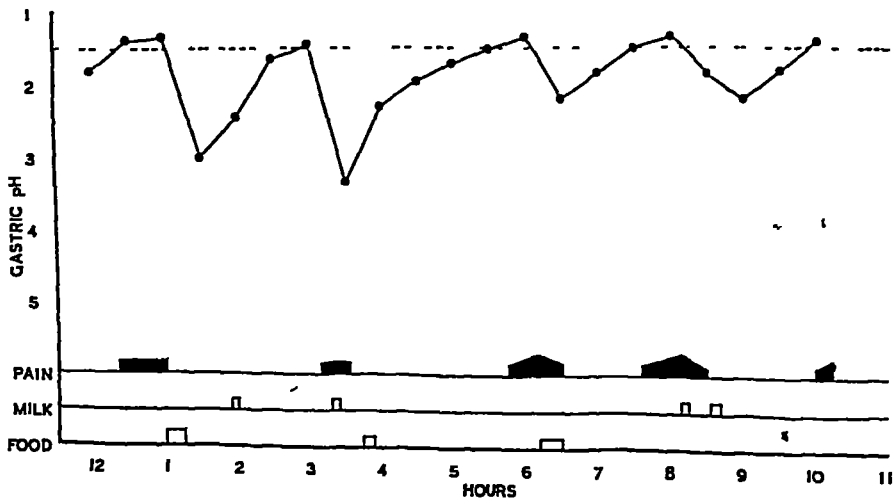
Fig 3 Subject C.A (duodenal ulcer) 23 11 44 Shows the pH of gastric samples removed by an indwelling Ryle's tube at half hourly intervals The patient recorded the times at which he removed the samples and the times of onset and subsidence of the pain The dotted line represents a pH of 1 50

rose to pH 1 4, declimng as the acidity fell after food and increasing as the
acidity rose to pH 1 25

(b) *Duodenal ulcer*   Records were obtained from three cases, pain
occurring at acidities greater than pH 1 5, pH 1 75, and pH 1 8   Fig 3 is an
example of a record which includes five periods of pain   In this intelligent
and co-operative patient, who kept careful records of his experiences, each
bout of pain began when the acidity of the stomach had risen to pH 1 5 or
beyond, and in each case was abolished by food and the consequent fall in
gastric acidity
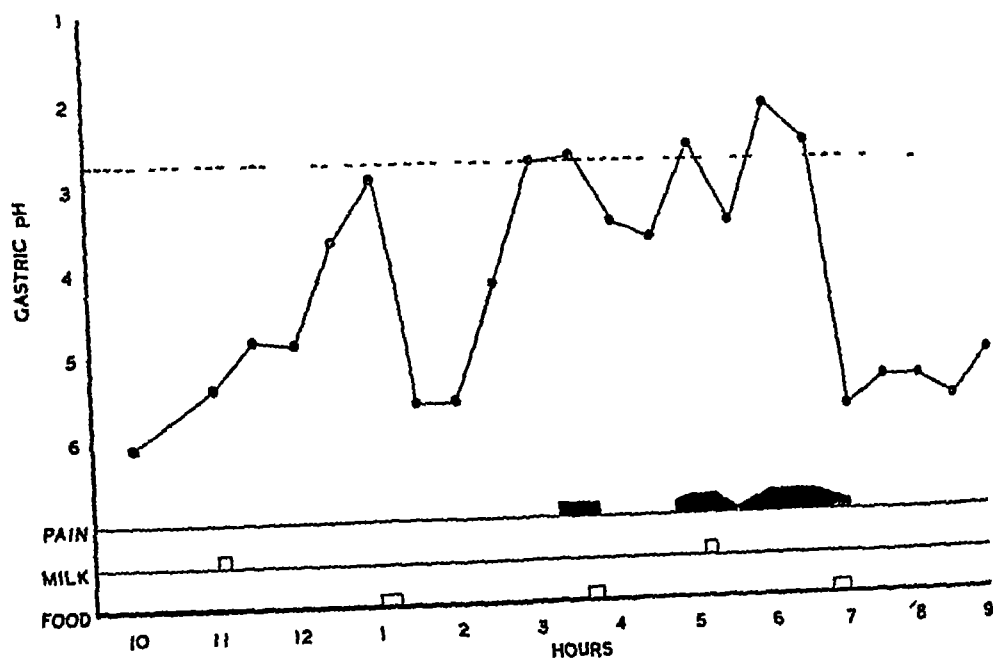
Fig 4   Subject R W , 6 1 45   A patient with a large gastrojejunal ulcer following partial
gastrectomy shown at operation to have penetrated the liver   Shows the pH of gastric
samples removed by an indwelling Ryle's tube at half hourly intervals over 12 hours, during
which the patient swallowed his meals at the usual time   Record of the times of removal
of the samples and of the times of pain was kept by the patient   The dotted line represents
a pH of 2 75

(c) *Anastomotic ulcer*   In the three cases investigated, the degree of
gastric acidity associated with pain was lower than was usual in gastric
or duodenal ulcer   Fig 4 is an example which includes two periods of pain,
occurring at pH values between 2 2 and 2 75   With lesser degrees of acidity,
pain was absent or, as in the second period of pain, had temporarily lessened

Comment   In interpreting these observations of which Figs 2, 3 and 4
are representative two points must be emphasised   First, where food was to be

taken at the time when a gastric sample was due, the gastric sample was invariably taken first and the food after, the lessening of gastric acidity which may legitimately be inferred to date from the time of ingestion of food is not shown until the next gastric sample   Second it will be shown in the next section that when the acidity of the gastric content is abruptly increased by the intragastric injection of HCl, pain does not occur at once, but only after an interval, conversely when the change is made abruptly from more acid to less acid, pain is not at once abolished, but takes some minutes to disappear. Bearing these considerations in mind it is clear from the examples given and from the other results we have obtained that pain in gastric or duodenal ulcer occurs when the stomach contents become more acid and is relieved as the acidity lessens, either spontaneously, or through the ingestion of food, further, pain nearly always occurs if the stomach contents attain a certain degree of acidity for a long enough time, and pain is always absent or declining when the gastric acidity is below a certain level for sufficiently long In general the agreement between more or less acidity and the waxing and waning of pain has been closest in duodenal ulcer, and rather less close in gastric and anastomotic ulcer   This again is readily accounted for by the variations of acidity in different parts of the stomach, for as Fig 1 shows the variation is greatest soon after ingestion of food, at the time when gastric ulcer pain occurs, and least at a long interval after food corresponding to the time of pain in duodenal ulcer   In anastomotic ulcer yet another factor may cause discrepancy, for the ulcer situated close to the stoma may be affected not only by the acid gastric content but also by the alkaline jejunal juice

Whilst, therefore, it seems that with ulceration of the stomach and duodenum the presence or absence of pain depends on the degree of acidity of the gastric content, it will be clear from inspection of the figures that the threshold of acidity necessary to provoke pain varies greatly from one patient to another   In general duodenal ulcer has shown the highest threshold, anastomotic ulcer the lowest with gastric ulcer intermediate, some cases of gastric cancer have a threshold still lower than is found in peptic ulcer Thus in the examples given the threshold of acidity necessary to provoke pain was approximately pH 1 5 in the duodenal ulcer (Fig 3) and in the gastric ulcer (Fig 2), while it was as low as pH 2 75 in the anastomotic ulcer (Fig 4)   A fall of threshold as the lesion progresses is best seen in gastric cancer where pain is at first not provoked by acidities less than say pH 1 6 (*see* Fig 8) and is easily abolished by alkali, and later is provoked by quite low acidities (pH 3) is nearly continuous and relieved with difficulty by alkali   With such irritable pain mechanisms it is highly likely that factors other than acid may suffice to excite

Production of pain by intragastric injection of acid

On days during which ulcer patients were experiencing spontaneous pain we have sought whether the pain can be reproduced by intragastric injection

of acid   The observations were made with the patient fasting, a Ryle's tube
being introduced, and the stomach emptied and washed out with tap
water *   If pain was present initially this procedure abolished it   200 to
300 c c of $\frac{N}{20}$ HCl were then introduced, a specimen taken, and the stomach
emptied again after 20 minutes and washed out with water   If no pain
resulted, a stronger solution $\frac{N}{15}$ or $\frac{N}{10}$ was introduced, but the greatest quantity
ever used was 300 c c $\frac{N}{10}$ acid   In about half of the cases the same quantity
of the same molar concentration of NaCl was introduced either before or
after the acid or between two injections of acid

The results were as follows —

(a) *Gastric ulcer*   In 13 cases of lesser curve gastric ulcer observed
during the days of pain, intragastric injection of acid reproduced the pain
in 11   The mean intragastric pH, that is the average of the pH just after
the introduction of acid and that at the time of aspiration is shown for 9 cases
in Table I   Pain began from 6 to 20 minutes (average 12½ minutes) after the
injection of acid and was relieved during the aspiration or gradually declined
during the succeeding 10 minutes   It was indistinguishable from that
spontaneously experienced   Injection of similar amounts of salt solution
did not produce pain   Of the two patients who failed to develop pain, one
vomited the tube and acid within a few minutes   The other, a male of 53,
with a gastric ulcer recurring after gastroenterostomy received 2 injections
of 200 c c , $\frac{N}{15}$ HCl and 200 c c $\frac{N}{10}$ HCl, the pH being respectively 1 50 and
1 58 just after the injections and 6 20 and 3 57 when, after 20 minutes, the
stomach was emptied of its deeply bile stained content   Later in the same
day he developed pain naturally and the gastric juice aspirated as the pain
was subsiding had a pH of 1 87   In this case it seems clear that the acid
injected was quickly neutralised by regurgitation through the stoma and the
requisite level of intragastric acidity was not obtained for a sufficient time
In two cases of prepyloric ulcer, pain occurred 4 minutes and 5 minutes after
intragastric, injection of 200 c c $\frac{N}{15}$ HCl, being relieved by emptying the
stomach in 4 minutes

(b) *Duodenal ulcer*   Of 9 cases of duodenal ulcer, acid injection
produced pain in 8 after 5 to 18 minutes (average 13 minutes) , the pain was
indistinguishable by the patient from that commonly experienced and was
relieved during the aspiration which lasted up to 10 minutes   The failure
occurred in a man of 38 whose pain the previous day had been relieved by
aspirating 130 c c (free acid 58 8 c c) from the stomach and who had pain
later on the day of the test , in him 200 c c $\frac{N}{15}$ HCl and 200 c c $\frac{N}{10}$ HCl both
failed to produce the pain, the pH at beginning and end of the 20 minute

* The tap water used was distinctly alkaline in reaction

TABLE I

Summarises data concerning the relationship between intragastric pH and pain

Name	Pain Period						Pain free Period	
	Natural Gastric Secretion				HCl or NaCl Injection		Natural Secretion	Acid Injection
	Pain present			Pain absent	Pain present	Pain absent		
	Lowest pH	Limits pH	Mean pH	Limits pH	Mean pH	Mean pH	Lowest pH	Mean pH
A Gastric Ulcer								
R Co	1 5	1 5 2 04	1 9	2 6 6 1	1 42	6 35	—	—
P H	1 24	1 24 1 81	1 5	3 0 7 5	1 41	3 1	1 23	1 42
R N	1 48	1 48 1 54	1 51	1 67 7 2	1 41	1 94	—	—
R C F	1 58	1 58 1 76	1 67	7 23 7 37	1 52	1 63	—	—
J.N	1 35	1 35 3 5	1 9	3 1	1 50	1 91	—	—
F.H	1 3	1 3 2 2	1 6	1 6 3 2	1 7	—	—	1 50
D L	1 53	1 53	1 53	1 75	1 33	—	—	1 15
W G	—	—	—	1 8	1 4	—	—	—
E M **	2 2	2 2 2 9	2 6	3 75-4 2	—	—	—	—
W C	1 55	1 55 1 75	1 65	1 4 3 5	1 14	—	—	—
B Duodenal Ulcer								
A.B	1 26	1 26 1 76	1 3	4 34	1 54	—	—	1 37
F.H	1 46	1 46 1 75	1 58	1 62 2 8	1 46	1 5	1 57	1 34
W Dr	1 39	1 30 1 91	1 70	—	1 68	2 85	—	—
W.De	1 12*	1 12 1 38	1 25	1 4 2 7	1 39	—	—	—
C.A.	1 35	1 35 1 45	1 4	1 7 5 2	1 2	—	1 32, 1 27	—
R Cl	1 15	1 15 1 3	1 22	1 4 2 8	1 2	—	—	—
T D	1 58	1 58	1 58	—	1 25	—	—	1 15
C Anastomotic Ulcer								
J.B	1 35	1 35 1 55	1 45	1 75 7 70	1 72	7 2	—	—
R W	1 6	1 6 2 75	1 9	2 65 6 1	1 2	—	—	—

* Histamine
** Patient had a gastroenterostomy

periods being 1 63 and 1 78 for the first and 1 41 and 1 51 for the second
The failure which we are unable to explain cannot be attributed to the acid
fluid failing to pass the pylorus for only 114 and 100 c c remained in the
stomach at the end of the two experimental periods of 20 minutes

(c) *Anastomotic ulcer* The usual ulcer pain was produced in one patient
9 minutes after injection of 300 c c $\frac{N}{20}$ HCl and 19 minutes after 300 c c $\frac{N}{15}$ HCl
and was in each instance relieved during aspiration In another patient
100 c c $\frac{N}{10}$ HCl produced violent pain beginning 1 minute after the injection
and unrelieved by emptying the stomach of 100 c c, but relieved after
20 minutes by sodium bicarbonate

(d) *Gastric cancer* In two cases of cancer of the body of the stomach,
verified at operation, and presenting with pain of the type here described,
pain was reproduced by intragastric injection of 200 c c $\frac{N}{20}$ HCl and
300 c c $\frac{N}{10}$ HCl in one case and 300 c cs $\frac{N}{10}$ HCl in the other Pain occurred
after a latency of 9 to 25 minutes and was abolished by alkali or aspiration

The relationship between the intragastric acidity during naturally occurring and during artificially produced pain

If intragastric acidity is in fact, the chief factor whose variation causes
the onset and subsidence of pain, then it is clear that the level of gastric
acidity at which pain naturally occurs should correspond with that at which
pain can be provoked by acid injection alone Table I summarises the
relevant data and some which will, later, receive attention Only those
figures in the table under the heading " Pain period " now concern us The
first four columns chiefly summarise the observations in which records of
gastric acidity and pain were made over 3 to 12 hours, as illustrated by
Figs 3, 4 and 5, and previously described Since, as has been noted in the
previous section, there is a time lag between the appearance of acid in the
stomach and pain, the acidities of specimens immediately before the onset
of pain have been omitted from the results in the fourth column (" pain
absent ") Where only one pH is recorded, only one sample of gastric
contents during or without pain was obtained The fifth and sixth columns
summarise the results of acid injection producing pain and acid or saline not
producing pain, the mean pH being arbitrarily taken as the arithmetic mean
of the figures obtained just after the injection of the fluid and at its withdrawal

From this table it may be seen that the mean pH during pain produced
by HCl injection was within the limits of pH recorded during natural pain
in 3 cases of gastric and 5 of duodenal ulcer, and more acid than any value
recorded during natural pain in 5 cases of gastric, 2 of duodenal and 1 of
anastomotic ulcer While there is thus a distinct tendency for the acid values
to be higher during artificially produced than during natural pain, we do not

consider that this invalidates the hypothesis that acid is the stimulus to pain, and for two reasons   Firstly, the tendency is most pronounced in gastric ulcer, in which as we have seen pain occurs at a time when variations in acidity between different parts of the stomach are most pronounced , sampling errors are thus to be expected in the observations on natural pain Secondly an interval elapses between the injection of acid into the stomach and the onset of pain , the exposure to acid was in general probably longer
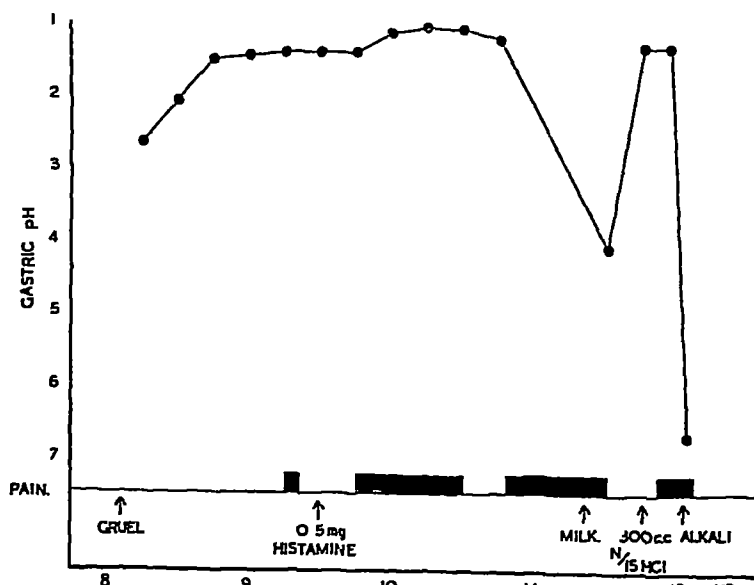
Fig 5   Subject W D (duodenal ulcer) 7 4 45   Shows the pH of gastric samples withdrawn by an indwelling Ryle s tube at quarter hourly intervals over 4 hours during which the patient recorded the times of onset and subsidence of his pain   Gruel given initially was followed by the spontaneous onset of pain increased by giving histamine 0 5 mg s c 1   Pain was relieved by milk and reproduced 6 min after introduction of 300 c c $\frac{N}{15}$ HCl

with natural secretion than with acid injection   This second consideration also applies to the three instances in which the mean acidity during acid injection unassociated with pain exceeded the mean acidity during natural pain   In view of the sources of error and the differences in conditions precise agreement between the two methods is not to be expected, and the fact that the same order of acidities are associated with pain in the two instances, may be accepted as at least not inconsistent with the view that acidity is a chief factor in the stimulus to pain

Observations during the pain-free period

In a number of cases we repeated the observations made during the pain period after the patient was symptomless and his ulcer believed to be healed

The second half of Table I shows that in 2 cases of gastric and 2 of duodenal ulcer, a similar degree of intragastric acidity occurred naturally without the patient experiencing pain   In 3 cases of gastric and 3 of duodenal ulcer in the symptomless period, injection of similar amounts of acid reduced intragastric pH to a similar level as before without causing pain   In addition 3 cases of gastric and 3 of duodenal ulcer, not tested in the pain period, experienced no pain with 200 to 300 c c $\frac{N}{10}$ acid in the symptomless period   Four normal subjects behaved similarly   However, absence of spontaneous pain in ulcer does not necessarily mean that the pain mechanism is totally insusceptible to acid   Thus in C A with a very refractory duodenal ulcer, pain was entirely absent during 34 days of strict diet and alkalis and nocturnal milk drip, at the end of this time 250 c c of $\frac{N}{10}$ acid produced typical ulcer pain in 12 minutes   Subsequently with a relaxation of his regime he had a return of natural pain and X-ray examination revealed a persistently active duodenal ulcer   In this case absence of pain may with some probability be ascribed to control of acidity rather than to insensitivity of the pain mechanism   Again in P H with a large lesser curve gastric ulcer, pain was reproduced 4 times by injecting 200 c c $\frac{N}{25}$ to $\frac{N}{15}$ HCl on 1 11 40 and 2 11 40   From 29 11 40 onwards he was free of pain   On 18 12 40 200 c c $\frac{N}{15}$ HCl produced typical pain relieved by aspiration and the following day X-ray revealed the ulcer reduced in size but still large   He remained pain free and on 12 2 41 200 c c $\frac{N}{15}$ HCl twice failed to produce pain though X-rays still revealed a definite niche, now greatly reduced in size in the same position as before   The niche subsequently disappeared and he remained sympton-free for at least five years   This case again illustrates that loss of spontaneous pain may precede the total loss of sensitivity of the pain mechanism to acid   It also illustrates that loss of sensitivity to acid may precede the complete anatomical healing of the ulcer   This was also shown in two further cases of very large chronic gastric ulcers in which some weeks after loss of spontaneous pain, acid injection was painless though X-rays in both and operation in one subsequently showed the ulcer to be of appreciable size

Comment

The observations here described provide strong evidence for the hypothesis that in gastric and duodenal ulcer the development and disappearance of pain are due to the rise and fall in acidity of the stomach content   Thus it has been shown that the development of naturally occurring pain is consequent on a rise and its subsidence on a fall of intragastric acidity beyond certain limits , that injection of hydrochloric acid solutions into the stomach provoke pain, while similar volumes of the same molar concentration of sodium chloride do not , and that the degree of intragastric acidity necessary to provoke pain is much the same whether the acidity arises as a result of the natural secretion of the stomach or through the experimental

injection of acid   That acidity is the natural stimulus to pain in peptic ulcer is therefore a generalisation that is in agreement with the facts here described, which confirm and amplify those previously recorded by Palmer (14)

Acidity is however, not the only factor in the production of pain   A degree of intragastric acidity, which in the presence of active ulceration is adequate to provoke pain, may occur naturally or be artificially induced without any resultant pain in ulcer patients during the pain free interval, and in normal subjects   Whether a certain degree of intragastric acidity will or will not provoke pain depends therefore on the state of the walls of the stomach or duodenum   Precisely what is this state of the stomach or duodenal wall that confers sensitivity to the acid stimulus is not at present entirely clear   A break in the continuity of the mucous membrane as in gastric, duodenal, or anastomotic ulcer, or in carcinomatous ulcer or erosive gastritis would account for the phenomena we have observed   Hardy (8) found that intragastric injection of acid would provoke pain in four cases of chronic appendicitis and four of cholecystitis in which no gastric or duodenal lesion was found at operation, and his findings may suggest that sensitivity is due, not to ulceration, but to some inflammatory state of the mucous membrane   We have, however, been impressed with the difficulty of excluding gastric or duodenal ulceration at operation , in a case with severe and repeated hæmatemesis, operation by Mr Dickson Wright disclosed no ulcer until the stomach was incised, when close inspection of the mucous membrane revealed an acute gastric ulcer 0 5 cm in diameter   Whatever the ultimate nature of the defect in the mucous membrane that confers sensitivity it 'is clear that in different patients such defects may be associated with very varying degrees of sensitivity to acid   As has previously been pointed out, in duodenal ulcer quite high degrees of acidity are usually necessary to provoke pain, while in anastomotic ulcer and gastric cancer a much lower value may be adequate

Brief consideration may now be given to the grounds on which previous workers have rejected the hypothesis that variations in intragastric acidity are the determining cause of the onset and subsidence of pain in peptic ulcer   The chief objection raised by Hurst (9) was his failure to reproduce pain by intragastric injection of acid in six cases of gastric ulcer confirmed at operation   This failure may have been due to the acid not having been left sufficiently long in the stomach to induce pain or to the ulcer having healed sufficiently for the pain mechanism to be insensitive to acid   That intragastric injection of physiological concentrations and amounts of HCl will reproduce pain in patients with gastric ulcer in whom pain is occurring naturally is abundantly clear from our results and those of Palmer (14) and Hardy (8)   Again the lack of agreement between the values for titratable acidity of the gastric content and the presence or absence of pain stressed by Hardt (7) and Christensen (6) finds explanation in the results here presented   The sources of error in estimating pH by titration, and in gastric

sampling have been mentioned   Even more important perhaps is the fallacy resulting from isolated gastric samples , for since increase and decrease in gastric acidity are followed by the development and disappearance of pain only after the lapse of a certain time, it is clearly compatible with the known facts for a single gastric sample taken during the presence of pain to be less acid than one taken during its absence, provided that the gastric acidity has undergone recent change as is not infrequent   Thus in the observations shown in Figs 2, 3 and 4, isolated samples could have been obtained showing higher acidities in the absence than in the presence of pain, and the conclusion reached that acidity was not the cause of pain , more frequent sampling prevented this error

Again Ryle (20) and others have pointed out that patients with severe pain from gastric ulcer may show a hypochlorhydria or even achlorhydria during a fractional test meal   Palmer has shown (13), and we have confirmed, that such patients may show much higher values for titratable acid during naturally occurring pain and after varied food than during a fractional test meal   Moreover, as has been clearly demonstrated here, the threshold of acidity to which the pain mechanism responds varies from patient to patient, and may be so low that pain is produced at levels of acidity very near the turning point of Topfer's indicator   That one subject secretes a highly acid juice and has no pain while another secretes a less acid juice and has pain with the properties here described means simply that the latter has an abnormality of the mucosa of the stomach or adjacent gut which makes it sensitive to acid   Finally, Hardy (8) failed to obtain pain in sensitive duodenal ulcer patients after histamine   Palmer (14) mentions examples in which the histamine secretion induced pain and an example of our own is shown in Fig 5

THE RELATIONSHIP BETWEEN PAIN AND GASTRIC CONTRACTION

Although the previous observations suggest that changes in intragastric acidity determine the appearance and disappearance of pain in ulcer of the stomach and duodenum, they do not show whether acid acts directly as a chemical irritant or indirectly through producing some change in the physical state of the walls of the gut or their attachments   Our evidence relating to the possibility that the stimulus to pain is a mechanical change will now be presented

Pain and intragastric pressure

Pain in peptic ulcer is continuous , it is therefore unlikely to be due to a discontinuous process such as repeated waves of contraction   If it is due to tension then it is likely to be due to a maintained contraction of the stomach or duodenum either general or local   To determine whether a generalised contraction of the stomach was involved we preferred to record intragastric pressure directly rather than by means of a balloon   Intragastric pressure

was recorded by connecting a Ryle's tube, indwelling in the stomach, to a vertical water manometer by means of rubber tubing incorporating a glass T piece, through which the whole system could be filled with water, and, by a suitable arrangement of pinch clips, fluid injected into or withdrawn from the stomach. The patient in each case was lying on his back in bed with the shoulders raised on two, and the head on three pillows, and the muscles as far as possible relaxed. The manometer was adjusted so that zero on the scale corresponded with the level of the umbilicus. The observations were made with the patient fasting, and the stomach was emptied and thoroughly washed out with tap water at the start. Each observation was divided into
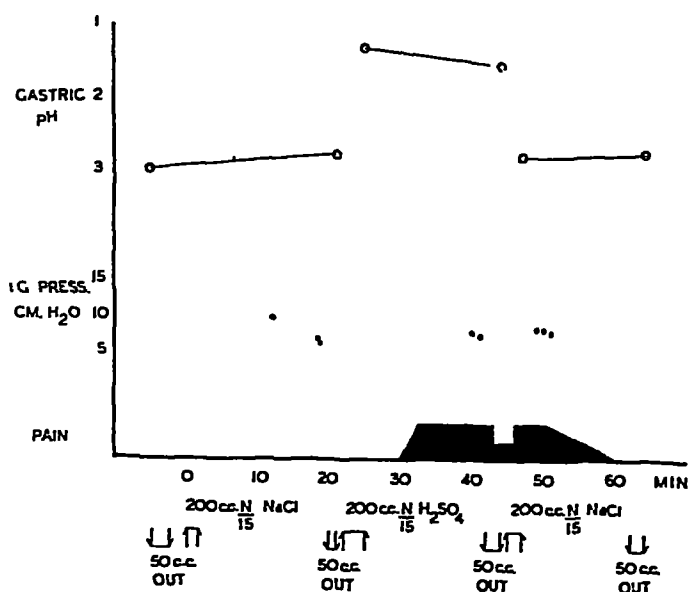
Fig 6. Subject W G (gastric ulcer shown at operation to have eroded pancreas) 3 7 41. Shows the intragastric pressure measured by a water manometer during three periods, in the first and third of which 200 c c $\frac{N}{15}$ NaCl was introduced into the empty stomach. In the middle period 200 c c $\frac{N}{15}$ H$_2$SO$_4$ was introduced and produced pain after 8 min., the pain being temporarily relieved by aspirating the stomach and increased again by the subsequent introduction of NaCl before finally disappearing. The pH of gastric samples removed at the beginning and end of each experimental period is shown.

at least two and usually three experimental periods, in one of which 200 to 300 c c of $\frac{N}{20}$ to $\frac{N}{10}$ HCl (H$_2$SO$_4$ in one experiment) was introduced into the stomach, left there for 20 minutes and then withdrawn, and in another the same volume of the same molar concentration of NaCl was introduced for the same length of time and subsequently withdrawn. Intragastric pressure is dependant on intraperitoneal pressure (largely due to the state of the anterior abdominal wall) and on gastric tone. In these experiments we have

attempted throughout to preserve the posture of the patient constant, to keep, as far as possible, intraperitoneal tension unchanged

The results of these observations are summarised in Table II and illustrated by Figs 6, 7 and 8   In gastric ulcer, pain was produced by acid in 6 patients   The mean intragastric pressure as Table II shows was in 5 cases a little lower during the period of pain, than it was during the control period when pain was absent   In one case the pressure during pain was a little higher than when pain was absent   This case, W G , a man of 51 with a chronic gastric ulcer found at operation to be floored by pancreas, was the subject of Fig 6 which presents other features of interest   During the first period in which 200 c c $\frac{N}{15}$ NaCl were introduced into the stomach, and in which pain was absent, frequent brief rises of pressure were observed in the manometer, presumably the result of gastric contractions , these brief rises are omitted from the record   In the second period 200 c c $\frac{N}{15}$ H$_2$SO$_4$ increased the acidity to pH 1 37, falling to pH 1 59 at the end of the 20-minute period During this period peristaltic waves were infrequent   Pain began 18 minutes after acid had been introduced and was eased by aspirating 50 c c from the stomach, but again increased by injecting 200 c c $\frac{N}{15}$ NaCl although the acidity had fallen to pH 2 87   The pain then declined to disappear 17 minutes after the NaCl injection (pH 2 79)   In one other case of peptic ulcer (A B , duodenal ulcer) and in the observation on a case of carcinoma of the stomach quoted on p 70, we have observed an increase in pain with intragastric injection of fluid which either left the acidity unchanged (A B) or reduced it In these three observations it seemed clear that the volume of the gastric content had an influence on pain, and it is of interest to note in Fig 6, the only observation of the three where it was recorded, that the intragastric pressure was not raised   In so far as volume of the gastric content is a factor in pain it does not therefore act by raising intragastric tension   We suggest as a tentative hypothesis that distension of the stomach may predispose to pain in ulcer by unfolding the rugæ and exposing the mucosal lesions more fully to the action of acid

Fig 7 illustrates one of two similar observations on a case of duodenal ulcer (proved at operation)   200 c c $\frac{N}{20}$ HCl produced pain in 15 minutes without any rise in intragastric pressure, the pain being abolished by emptying the stomach   200 c c $\frac{N}{10}$ NaCl left in the stomach for 26 minutes produced no pain, but as in the observation previously described, and indeed as we have usually observed, contraction waves, absent or inconspicuous during the acid period, now became frequent, and are shown in the figure After emptying the stomach 200 c c $\frac{N}{10}$ HCl were replaced and followed by one transient peristaltic wave and after 5 minutes by pain which was relieved by aspiration   The intragastric pressure in *this case* of duodenal ulcer
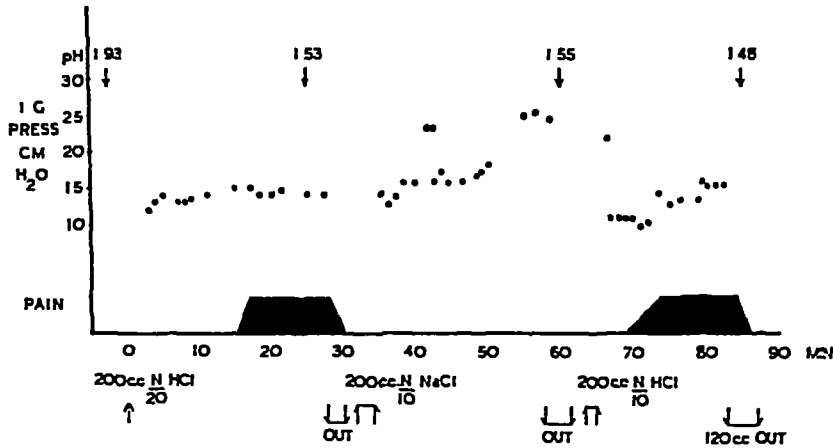
Fig 7 Subject F W (duodenal ulcer) 28 2 40 Shows the intragastric pressure measured by a water manometer during three periods in the first of which 200 c c $\frac{N}{20}$ H Cl and in the third 200 c c $\frac{N}{10}$ HCl were introduced into the empty stomach Pain produced in the first period 15 mins and in the third 5 mins after the introduction was relieved in each case by aspiration In the middle period the introduction of 200 c c $\frac{N}{10}$ NaCl produced no pain
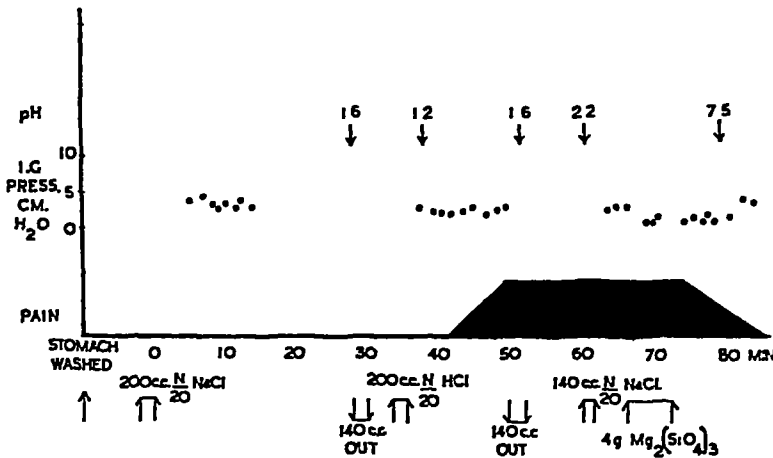
Fig 8 Subject J S 16 8 41, large ulcerating cancer of the fundus demonstrated at operation Shows the intragastric pressure measured by a water manometer during three periods in the first of which 200 c c and in the third 140 c c of $\frac{N}{20}$ NaCl were introduced into the empty stomach In the middle period 200 c.c $\frac{N}{10}$ HCl were introduced and produced pain after 7 mins no relief being obtained by aspiration or introduction of NaCl Pain was finally relieved 16 mins after giving 4 g magnesium trisilicate

was higher than in the other cases summarised in Table II, but we do not attribute any significance to this finding in a single case

Fig 8 shows the observation on a case of carcinoma of the fundus of the stomach   Here again pain was absent during the period in which 200 c c $\frac{N}{20}$ NaCl was left in the stomach but began 8 minutes after injection of 200 c c $\frac{N}{20}$ HCl , the pain was not relieved when this was aspirated, nor altered when the volume removed (140 c c) was replaced by $\frac{N}{20}$ NaCl which reduced the acidity to pH 2 2   The pain was slowly relieved by injecting magnesium trisilicate into the stomach (pH of gastric content 7 5)   Pain was again unassociated with significant change in intragastric tension

These observations, though not numerous, are conclusive in showing that pain in gastric ulcer, duodenal ulcer and gastric cancer is not due to general increase in tone in the wall of the stomach   In fact the stimulus used to provoke pain, acid, produced in 7 out of the 8 observations a slight fall in intragastric pressure which was not altered by the subsequent onset of pain , also a diminution in the frequency of peristaltic waves again uninfluenced by the occurrence of pain   In the one case of duodenal ulcer, the peristaltic waves occurring frequently with NaCl were recognised by a coincident transient feeling of fullness by the patient and the less frequent waves occurring during the pain provoked by HCl were accompanied by a momentary increase in pain   As others have found, therefore, peristalsis may modify pain , it is not its cause

TABLE II

The relationship between intragastric pressure and pain

Patient	Mean Intragastric Pressure (cm H_2O)	
	With pain	Without pain
A Gastric Ulcer		
L C	7 5	9 0
W G	8 2	7 5
F C	3 5	4 0
R F	6 5	7 5
J N	3 2	4 5
B Duodenal Ulcer		
L W	12 0	13 0
	14 5⎫ 13 0⎭	16 0
D Carcinoma		
J S	2 0	3 0

X-ray examination of the stomach and duodenum

In these observations made on the fasting patient, pain was produced by introducing into the stomach through a Ryle's tube 200 or 300 c c barium sulphate emulsion containing HCl to a concentration of $\frac{N}{10}$ The patient was screened and films taken at intervals   The stomach was subsequently emptied and a similar quantity of the same strength of barium emulsion without acid introduced and the examination repeated, control films being taken at similar times after introducing barium as in the previous period In some observations the procedure was reversed the neutral barium being introduced first   The patient remained erect throughout   Seven patients with gastric ulcer, 2 patients with duodenal ulcer, 1 patient with an anastomotic ulcer and 2 patients with carcinoma were so examined, and the appearances with and without pain compared   In addition 2 normal subjects and 3 patients with duodenal ulcer who did not get pain from the acid barium were examined   The differences between the behaviour and appearance of the stomach containing neutral and acid barium were similar over the whole group   In all subjects gastric peristalsis was much more active and the stomach emptied more quickly with neutral than with acid barium   The tone of the stomach appeared better with neutral than with acid barium The duodenal cap sometimes seemed larger with neutral and sometimes with acid barium   In none of these patients did we see localised contractions of the stomach or duodenum during the period of pain that were not present without pain   Thus in the patients with gastric ulcer, both the appearance of the crater and the spasm of the greater curvature opposing it were unaltered during the period of pain   With such contrasting rates of peristaltic activity and emptying it was difficult to compare accurately the condition of the pylorus , but in some of the gastric ulcers and in one of the two duodenal ulcers Dr Blair was able to satisfy himself from the ease with which barium could be expressed into the duodenum that no pylorospasm was present during the period of pain

These observations have satisfied us that local contraction of the gut is not the cause of pain in ulcer of the stomach and duodenum   Such departures from the normal as we have observed during the period of pain have not been due to pain but to the stimulus, acid, used to provoke it, as is evident from the similar changes seen in normal subjects and ulcer patients without pain

A word may appropriately be said here on the effect of acid on the motor activity of the stomach   The observations on intragastric pressure and the radiological examinations have consistently shown a reduction in peristaltic activity of the stomach into which hydrochloric acid in concentrations of $\frac{N}{20}$ and $\frac{N}{10}$ has been introduced   The radiological examinations have also shown a slowing in the rate of gastric emptying   The same conclusion is reached by comparing the amounts of fluid that can be with-

drawn from the stomach after similar volumes of the same molar concentrations of HCl and NaCl have been introduced for similar periods In observations made on 7 patients the volume withdrawn was in 6 greater with HCl than with NaCl   For example in case P H , gastric ulcer, 85 and 66 c c were withdrawn 22 and 23 minutes after introducing 200 c c NaCl solution ,   144 and 140 c c were withdrawn 21 and 22 minutes after introducing 200 c c of HCl solution   Hydrochloric acid in the concentrations here used, which are within the range of those occurring naturally in the stomach, clearly reduces the motor activity of the stomach and prolongs its emptying time

Comment

These observations agree with the considerable body of evidence already presented by other workers in demonstrating that neither increased general tone, nor local contraction nor peristalsis are the cause of pain in peptic ulcer   Nor is sagging of the relaxed stomach with consequent traction on its superior attachments the cause of pain, for we have been unable consistently to modify the pain produced by acid by changing the posture of the patient on a swing couch from the head up through the horizontal to the head down position   The pain indeed has nothing of the properties which characterise other pains of mechanical origin in that it is uninfluenced by purely mechanical factors such as influence, for example, the histamine headache   This failure to find any evidence of a mechanical factor responsible for ulcer pain leaves as the more probable the alternative explanation, namely that acid produces pain by acting chemically as an irritant

GENERAL DISCUSSION

The evidence presented in this paper offers strong support to the hypothesis that pain in ulcer of the stomach and duodenum results from exposure of a defective mucous membrane to a certain level of hydrogen ion concentration for a sufficient time   Disagreement with this view seems to have been based on imperfect collection of data, and on speculative considerations   The explanation advanced for the mechanism of ulcer pain is in full accord with the well known relationship between pain and the ingestion of food   Thus, in gastric ulcer the peak of intragastric acidity is usually reached between 1 and 1½ hours after food, after which acidity declines , while in duodenal ulcer intragastric acidity usually reaches a peak 2 hours or more after food and remains at or about this level until food is again ingested   In both these conditions the time intensity curve for pain closely simulates that of intragastric acidity   Again the characteristic relief of pain by alkali, by food, and by vomiting is simply explained on the acid hypothesis   In fact we would go so far as to suggest that pain occurring some time after food and relieved by alkali, by food or by vomiting always arises as a consequence of the action of acid gastric juice on a lesion of the

stomach or of the gut in direct continuity with the stomach, such as the duodenum or, in surgical anastomosis, the jejunum , and that this is in general true whatever the site of the pain   Instances of unusual reference of pain will be given in the next paper   The conception that pain with the above characteristics may result purely from some motor or secretory disturbance of the stomach excited reflexly from a lesion of the appendix or gall bladder has, we think, arisen as a consequence of the difficulty of recognising by X-rays small lesions of the mucous membrane of the stomach and duodenum The confusion should be dispelled by the increasing frequency of gastroscopic examination and by improvements in radiological technique

The few observations recorded in Table I, and the records of Brown and Dolkart (3) suggest that intragastric acidity is not greatly different during the periods of relapse and remission which are so conspicuous a feature of the natural history of peptic ulcer   It seems probable, therefore, that the difference between the patient when he has frequent severe pain and when he has none is not due to the more extreme acidities reached, but to the more vulnerable state of the gut wall in the pain period

Although it seems clear that acid is ordinarily the agent which excites pain in simple or malignant ulcer of the stomach and adjacent gut, it should be stated at once that there may be other agents or mechanisms causing pain from these sites   We have encountered patients with gastric cancer and other conditions in which the gastric juice was found to be persistently alkaline or neutral to litmus and in which there was reason to believe that pain came from the stomach   The mechanism by which pain arises in such conditions has not been investigated and they are mentioned here merely so that they should not be confused with the pain considered in this paper

The precise nature of the mechanism by which acid excites pain when a gastric or duodenal lesion is present will be more fully considered in the next paper which deals with the site of the pain nerve-endings concerned It may be said, however, that there are clearly two possibilities, namely that acid may act directly on pain nerves or it may act indirectly by injuring the cells of exposed tissue from which is released a substance which itself is the stimulus to which pain nerve endings respond   Peptic digestion of the exposed tissue is, we think, unlikely to be a factor of importance in the production of pain, because the optimum pH for peptic digestion is about 1 9, whereas as we have seen from the level of acidity at which pain occurs is in many cases considerably higher (see for example Fig 2 and 3)   Whether it acts directly or indirectly acidity itself is probably the irritant causing ulcer pain

The hypothesis developed in this paper concerning the mechanism of pain in peptic ulcer has applications to practical therapeutics   The occurrence of pain having the characteristics described in this paper implies firstly that the mucous membrane of stomach or adjacent gut is defective,

drawn from the stomach after similar volumes of the same molar concentrations of HCl and NaCl have been introduced for similar periods In observations made on 7 patients the volume withdrawn was in 6 greater with HCl than with NaCl For example in case P H, gastric ulcer, 85 and 66 c c were withdrawn 22 and 23 minutes after introducing 200 c c NaCl solution, 144 and 140 c c were withdrawn 21 and 22 minutes after introducing 200 c c of HCl solution Hydrochloric acid in the concentrations here used, which are within the range of those occurring naturally in the stomach, clearly reduces the motor activity of the stomach and prolongs its emptying time

Comment

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GENERAL DISCUSSION

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the stımulus to whıch paın nerve endıngs respond   Peptıc dıgestıon of the
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The hypothesıs developed ın thıs paper concernıng the mechanısm of
ın ın peptıc ulcer has applıcatıons to practıcal therapeutıcs   The
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stly that the mucous membrane of stomach or adjacent gut ıs defectıve,

for example by reason of a single ulcer or multiple erosions, and secondly that intragastric acidity is exceeding the level at which acid is acting as an irritant upon the living cells of the gut wall   It may be inferred, therefore, that unless the regime adopted in the treatment of ulcer keeps the patient free from pain it is not maintaining conditions in the foregut which are conducive to the repair of the lesions of its wall, an inference which is broadly in accord with experience

SUMMARY

1   The relationship between the development and subsidence of pain and changes in intragastric acidity has been investigated in patients suffering from peptic ulceration of the stomach, duodenum and surgical anastomosis between stomach and jejunum and in a small number of cases of gastric cancer   Samples were obtained by gastric intubation and their pH determined by the double quinhydrone electrode

2   The hydrogen ion concentrations of samples simultaneously withdrawn from the body and pyloric end of the stomach are not identical, the descrepancy is greatest soon after the ingestion of food

3   Naturally occurring pain is relieved by emptying the stomach, it can be reinduced by returning the gastric contents, it is not reinduced if the gastric contents are neutralised before being returned to the stomach

4   Repeated sampling of the gastric contents shows that naturally occurring pain is closely related to changes in intragastric acidity   Pain tends to occur if the hydrogen ion concentration exceeds a certain level for a long enough time, and to disappear if the acidity falls much below this level

5   During the period when the patient is subject to naturally occurring pain intragastric injection of 200 to 300 c c of $\frac{N}{20}$ to $\frac{N}{20}$ HCl induces pain after a latent period of about 10 minutes   The pain is relieved in a few minutes by withdrawal of the acid and is not induced by similar amounts of the same molar concentration of NaCl

6   In the pain free period and in normal subjects injection of acid does not induce pain

7   There is a close relationship between the levels of intragastric acidity associated with naturally occurring pain and the levels associated with pain induced by intragastric injection of HCl

8   It is concluded therefore that in a general way pain in peptic ulceration is due to the action of a certain degree of acidity for a certain time on a gastric or intestinal wall whose mucous membrane is defective

9 The intragastric pressure during pain induced by HCl solutions is not higher but is usually slightly lower than in the absence of pain with similar volumes of NaCl solutions introduced into the stomach

10 Radiological examination has revealed during the period of pain no localised contractions of stomach or duodenum, that were not present when pain was absent

11 It is concluded that pain in peptic ulcer is not due to general or local contraction of the stomach or duodenum Pain is due to the chemical action of acid on a defective gut wall

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OBSERVATIONS ON THE MECHANISM OF PAIN IN ULCER OF THE STOMACH AND DUODENUM

PART II — THE LOCATION OF THE PAIN NERVE ENDINGS

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In the previous paper (3), confirming and amplifying the results of Palmer (21, 22, 23), evidence has been presented that in ulcer of the stomach and duodenum pain is due to the action of a sufficient concentration of hydrogen ions for a sufficient time on a defective gastric or duodenal wall   Since search failed to reveal that acid produces pain through a mechanical change in the gut or its attachments it may be presumed to act through a chemical stimulation of pain nerve endings   The location of these nerve endings will be considered in this paper

The application of dilute hydrochloric acid to an ulcer of the skin is painful, while the same solution applied to normal skin is not   It may not therefore seem surprising that the attainment of a certain acidity of the gastric content should give rise to pain when the surface of the stomach or adjacent gut is ulcerated and yet be painless when the mucous membrane is intact   Nor would it be surprising to find that the degree of acidity necessary to produce pain should depend on the extent and intensity of the inflamed area   The simple explanation that pain in gastric and duodenal ulcer is due to direct stimulation by acid of pain nerve endings in the ulcerated areas would fit the facts recorded in the previous paper   But it cannot be accepted without further consideration because of the doubt as to whether the wall of the gut contains pain nerves at all   For most of the somatic structures known to be sensitive to pain can be shown to give rise to pain when directly stimulated mechanically   In the case of the stomach and duodenum the evidence is conflicting and will now be considered

The pain sensibility of the stomach and duodenum to mechanical stimulation

Animal experiment is indecisive as to whether afferent nerves pass from the stomach and duodenum to the central nervous system

Thus McSwiney and Suffolk (18) obtained reflex dilatation of the pupil in chloralosed cats by inflating balloons in stomach or duodenum, and they were able by this method to identify the spinal roots by which the afferent fibres entered the cord   On the other hand Lewis and Kellgren (15) were able constantly to obtain in the spinal cat reflex contraction of the right rectus abdominis muscle from pinching the mesentery of the duodenum, which includes pancreas, but none from the duodenum itself   Whatever the outcome of such experiments it is clear that in view of species differences the final evidence must be obtained in man   The chief data come from the experience of surgeons who have tested the sensibility of the contents of the abdominal cavity   The most comprehensive study is that of Lennander (9, 10), who, operating under morphine and anæsthetisation of the skin with cocaine, observed the stomach to be quite insensitive to cutting, burning, pinching, clamping and pricking   It is true that, in the three cases where his observations are fully described, a general anæsthetic had to be used in the early stages, but the patient was awake and answering questions before two gastroenterostomies and one pylorectomy were performed, and in at least one case pain arising from traction on the mesentery was felt   Lennander's contention that the hollow abdominal viscera are totally insensitive to pain was confirmed by Mackenzie (17) who stated that in some cases he operated without any anæsthetic, but he gave no details concerning the stomach In the following case our own observations agree closely with those of Lennander

On 3 12 45 a gastrostomy was performed by one of us (G L W B) on C L , age 56, suffering from bronchial carcinoma, obstructing the œsophagus   Heroin 0 01 g was injected subcutaneously 2 hours before operation   The anterior abdominal wall was infiltrated with 100 c c ½% novocaine and adrenaline over an area extending outward and above to the left costal margin, inward to a line 1″ to the right of the midline and below to a transverse line at the level of the umbilicus   Approach was by a left epigastric transrectus incision

Incision and retraction of rectus sheath and muscle caused moderate pain   Incision and stretching of the extra peritoneal tissue caused severe pain   The abdomen being opened, the stomach was withdrawn gently   Any more than gentle traction on the mesentery of the stomach caused severe pain, nausea and tachycardia

The small incision in the stomach wall was made on the anterior wall of the pyloric antrum midway between the greater and lesser curvatures, about 3″ from the pylorus   Pinching the stomach, the insertion of sutures, cutting the stomach for the insertion of the tube, and the tension of the wall consequent on stitching the stomach round the tube were entirely painless

0 5 c c N/10 HCl poured on the intact mucous membrane and left for two minutes caused no pain   0 5 c c N/10 HCl applied to the cut surface of the stomach, and left for the same time produced no pain

Subsequent to the application of HCl, the patient felt pain during the necessary manipulations for insertion of the tube and for closing the abdomen, but again, traction on the mesentery or handling of extraperitoneal tissue was necessary for the production of pain

That the stomach is insensitive is the very general view of surgeons who have experience of operating under local anæsthesia   On the other hand,

Wolff and Wolff (28) in their studies on a male subject with a permanent gastrostomy opening found that pinching or applying a faradic current to normal gastric mucosa was painless but that when the mucosa was inflamed these stimuli were painful

Morley (19), operating after infiltrating the abdominal wall with procaine, noted that picking up the inflamed stomach wall adjacent to a prepyloric ulcer was painless in one case, and in another that seizing a duodenal ulcer with toothed forceps and squeezing ulcer between finger and thumb aroused no sensation   On the other hand Dragstedt and Palmer (4), operating with procaine anæsthetisation of the abdominal wall on a case of duodenal ulcer, shown shortly before operation to be acid sensitive, found that on gently rubbing the serosa over the puckered scar on the anterior surface of the duodenum, 1 cm distal to the pylorus, pain similar to ulcer distress was experienced and persisted after rubbing was stopped   Traction on the duodenum also gave pain, which was relieved by injecting 20 c c 5% sodium bicarbonate solution through a hypodermic needle into the lumen of the pylorus, although traction was maintained   20 c c of 0 5% HCl injected into the lumen 5 minutes later produced pain almost immediately which was partly relieved by injection of sodium bicarbonate   They observed also contraction waves passing across the pyloric antrum unassociated with pain and a contraction ring of the duodenum, distal to the ulcer, associated with momentary cramping pains

It is by no means easy to explain these very divergent findings of careful and experienced observers , it may be suggested that Palmer and Dragstedt's case differed from Morley's two in that the ulcer was already being stimulated by the acid chyme nearly to the point of pain, as the relief occasioned by NaHCO$_3$ suggests, with a consequent lowering of threshold to mechanical stimuli , for a very similar state of affairs has been observed in inflamed or injured skin by Lewis and Hess (14)   A precisely similar difference of opinion exists as to whether pain can be elicited from the region of the ulcer by pressure applied through the abdominal wall in the intact subject

Mackenzie (17) maintained that tenderness elicited by pressure on the abdominal wall came chiefly from the muscles and that pain and tenderness were localised to an area which did not correspond to the position of the ulcer as seen at operation or autopsy   Hurst (8), as a result of his experiences during X-ray examination of the stomach and duodenum, subsequently concluded that tenderness was of two kinds, visceral tenderness elicited by deep palpation, localised to the actual ulcer crater and likewise moving with change of posture, and reflex tenderness arising from the abdominal wall, constant in position and elicited by lighter palpation   Hurst attributed deep tenderness to the inflamed serosa overlying the ulcer being sensitive to pressure   Hurst's observations on deep tenderness were confirmed by Morley and Twining (20), who, in 24 cases of gastric and duodenal ulcer, marked the position of deep tenderness in the erect, supine and two lateral positions and subsequently X-rayed the patient   The position of the point

of deep tenderness changed with posture and was found to coincide with the actual ulcer crater in each position   Influenced by Morley's operative finding that the exposed ulcer was insensitive to mechanical stimulation even when deep tenderness could be elicited before anæsthetising the abdominal wall, they supposed the moving tenderness arose from the parietal peritoneum sensitised by contact with the inflamed ulcer   With this interpretation we cannot agree, doubting the ability of the momentary presence of an inflamed serosa in contact with the parietal peritoneum to confer on the latter a lowered threshold to pain from pressure   Moreover, in Morley's case, J J, in which deep tenderness was observed to be present immediately before operation, and to move considerably with change in position (Morley's Fig 15 (19)), it seems that the ulcer was not actually in contact with the anterior abdominal wall for in his description of the operation he states " When the liver margin was gently drawn up by a finger a large stellate ulcer in the anterior wall of the duodenum was exposed to view "   But close correspondence of deep tenderness and ulcer crater is striking enough to suggest that pain can be elicited from the ulcer crater or its immediate neighbourhood by pressure   If it is possible to elicit pain from the ulcer by a mechanical stimulus when it is active it is clearly conceivable that a chemical stimulus may also do so

The difficulty in accepting deep tenderness as a proof of the sensibility of the ulcer area to pain lies in the fact that the stimulus used to elicit pain, namely digital pressure, affects not only the ulcer and its immediate adnexa but also the anterior and the posterior abdominal walls   The intervention of one of these structures can be eliminated experimentally, for the anterior abdominal wall can be completely anæsthetised by injecting procaine around the intercostal nerves

The effect on tenderness of anæsthetising the anterior abdominal wall

Our first experiments were made with local infiltration anæsthesia of the abdominal wall   In one case of carcinomatous ulcer and one of peptic ulcer of the stomach, infiltration of the subcutaneous tissue with 2% procaine, rendering the skin anæsthetic and analgesic failed to affect the tenderness, but deeper infiltration of the rectus abolished it   Although, therefore, it seemed that tenderness originated in the rectus or its sheath, we could not exclude the possibility that some of the anæsthetic might have entered the peritoneal cavity

We therefore proceeded to produce regional anæsthesia by injecting 1 to 2% procaine with adrenaline around the intercostal nerves in the anterior axillary line from T 5 to T 11 inclusive on one or both sides   The amount of anæsthetic used varied from 15 to 75 c c   In each case the total tender area was mapped out, and the site of maximum tenderness to deep palpation marked in ink on the skin of the supine subject before the nerves were injected   The course of analgesia and anæsthesia and of rectus paralysis was observed at intervals and any change in tenderness noted

In some of these patients, on the same or a closely preceding or succeeding day, the point of maximum tenderness to deep palpation was again determined and covered with a radio-opaque marker after the stomach had been filled with barium emulsion , a film was then taken to show the relationship of the point of maximum tenderness to the ulcer The point of maximum tenderness remained constant from day to day in all the patients we have observed

The results of these observations are summarised in Table I Of three patients with gastric ulcer, one of whom had a gastro-enterostomy, complete analgesia of the epigastrium was obtained from umbilicus to ensiform cartilage in 2, and in both tenderness to deep palpation was completely abolished In one of these pain had been produced before the intercostal block by intragastric injection of $\frac{N}{10}$ HCl, and this pain disappeared during the course of the analgesia In the third case with a very large penetrating ulcer of the lesser curve only partial analgesia was obtained on two occasions and in each case the tenderness, which was pronounced, remained unaltered A marker placed on the tender spot covered the lower edge of the ulcer crater on the X-ray film

In 9 out of 10 observations on 9 cases of duodenal ulcer, tenderness was completely abolished to deep palpation In 5 of these observations pain was absent throughout In one, spontaneous pain present at the outset disappeared during the observation, although the acidity of the gastric contents taken after the cessation of pain was pH 1 37, an adequate acidity in this patient to initiate pain In one, spontaneous pain present at the outset remained when analgesia had developed and tenderness had gone, later becoming less and chiefly above its usual site In 2 others, pain produced initially by intragastric injection of acid disappeared with tenderness though acidity remained high In the three cases where the point was enquired into, the point of maximum tenderness lay at some distance from the duodenum The following protocol is illustrative

C.A Duodenal ulcer 19 11 44

6 30 p m Complains of aching pain one inch above and just to the right of umbilicus continuous since 5 p m and gradually increasing Exquisite tenderness over area, two inches in diameter one inch above and to the right of the umbilicus Less tender in diffuse area above this both to the right and to the left of the mid line Tight contraction of the right rectus abdominis muscle

6 45 to 7 15 p.m 60 c c 1 5% novocaine with adrenaline injected around intercostal nerves T5 to 12 on both sides in mid axillary line

7 15 p m Analgesia to pin prick extending above to 5th and 6th costal cartilages and below to two inches and one inch beneath the umbilicus on the right and left sides respectively Stroking analgesic area felt as rubbing sensation.

7 20 p m. Complete paralysis of upper rectus on both sides Complete anæsthesia to cotton wool over area of analgesia Pain has now gone and is replaced by sensation of heaviness in epigastrium. Tenderness to moderate pressure abolished

7 30 p m No pain , analgesia and anæsthesia persist No tenderness even on heavy pressure with fist through epigastrium, and palpation of posterior abdominal wall, through paralysed rectus

7 50 p.m Analgesia and anæsthesia persist Tenderness still unobtainable

22 11 44 Tenderness persists in same position though it has been less pronounced since intercostal block area marked with radio opaque wire in erect position Barium meal shows deformed irritable duodenal cap two inches above the tender area

TABLE I

The effect of regional anaesthesia of the abdominal wall on tenderness

Patient	Position of ulcer	Position of tenderness relative to ulcer	Pain	Intercostal nerve injection with novocain			
				Anaesthesia	Analgesia	Effect on tenderness	Effect on pain
E M	Lesser curve Gastroenterostomy	Over anastomosis	Same day	Complete R and L	Complete R and L	Abolished	No pain throughout
W G	Penetrating lesser curve	Corresponds	Same day Severe $\frac{N}{10}$ HCl	Partial Partial	Partial Partial	Unaltered Unaltered	No pain throughout Severe pain relieved
D L	Lesser curve	3" below and to R	Severe $\frac{N}{15}$ HCl	Complete R and L	Complete R and L	Abolished	Severe pain abolished Gastric pH 1 44
M K	Duodenal	2" from duodenum	Previous day	Complete R and L	Complete R and L	Abolished	No pain throughout
J W	Duodenal	Corresponds	Previous day	Partial	Complete R	Remains, diminished Abolished	No pain throughout
H L	Duodenal	2" above and to R	—	Complete R	Complete R	Abolished	No pain throughout
R Co	Duodenal	2" above and 1" to L	Same day	Complete over	tender area	Abolished	No pain throughout
J A	Duodenal	—	None for 2 days	Complete R	Complete R	Abolished	No pain throughout
C A	Duodenal	2" below ulcer	Present	Complete	Complete R and L	Abolished	Pain abolished Gastric pH 1 37
R Cl	Duodenal	—	Present $\frac{N}{10}$ HCl	Complete L	Complete L	Abolished L	Pain abolished Gastric pH 1 34
J D	Duodenal	—	Present $\frac{N}{10}$ HCl	Complete R	Complete R	Abolished	Pain abolished Gastric pH 1 35
J G	Duodenal	—	Pain severe	Complete L Partial R	Complete L Partial R	Abolished	Pain unrelieved— later goes
W C	Gastric and Duodenal	2 to L of D U Supine 1 above anc to L erect	Previous day	Complete R Partial L	Complete R Partial L	Abolished	No pain throughout
J B	Anastomotic	—	Pain $\frac{N}{20}$ HCl	Partial R and L	Partial R and L	Abolished except point in analgesic area	Pain reproduced by HCl
H H	Carcinoma Rody	—	Same day	Complete R and L	Complete R and L	Abolished	No pain throughout

The remaining patient, J W , was tender over the right rectus midway between umbilicus and xiphisternum   Injection of the right intercostal nerves T5 to 11 inclusive produced complete analgesia to pin prick but incomplete loss to touch extending from umbilicus to ensiform, and tenderness, though diminished, could still be elicited by deep pressure through the analgesic skin   In the erect position a marker placed over the point of maximum tenderness overlay the deformed and irritable duodenal cap as visualised by X-ray

In one case of an active gastric and inactive duodenal ulcer (operation finding) and in one of carcinoma of the body of the stomach tenderness was abolished by intercostal block

We conclude from these results that in most cases of gastric and duodenal ulcer, much if not all of the tenderness to palpation is elicited from the structures of the anterior abdominal wall   This conclusion is reinforced by control observations on 4 cases (D L , R Cl , J D , and W C) in which injection of the same quantity of the same solution of procaine and adrenaline subcutaneously over the ribs T 5 to T 11 produced no analgesia of the epigastric skin, no muscular paralysis and no change in epigastric tenderness , the abolition of tenderness in the observations cited therefore must have been due not to the procaine itself but to its interrupting conduction through the intercostal nerves   This conclusion also agrees with the lesser number of cases in which we have shown that the tenderness and ulcer do not correspond in position   It harmonises with our repeated failure to demonstrate that tenderness shifts with the posture of the patient and agrees with the conclusions reached by Bolton who, using only light palpation, found tenderness to be constant in position and not to overlie the ulcer crater

We have been able to confirm this conclusion by an observation of a different kind in one patient with an epigastric hernia 9 cm + 6 cm , following a midline incision for perforated gastric ulcer, and who had recurrent attacks of pain due to anastomotic ulcer following partial gastrectomy , we observed that during one of these attacks tenderness was maximal in the upper part of the right rectus close to the costal margin , deep palpation of the epigastrium between the recti was painless , inserting the thumb through the hernia and nipping the rectus between finger and thumb was painful when the upper right, but not when the upper left, rectus was so seized

We were interested to observe that in many of these cases with regional anæsthesia, tenderness did not return for 1, 2 or 3 days, although the sensation in the skin had recovered completely in 2 or 3 hours   In one case the tenderness, abolished by intercostal block, did not reappear during the whole of the patient's 4-week stay in hospital   This new observation may be set alongside the old to which Ryle refers, that tenderness often persists many days after the patient has, in response to treatment, ceased to feel

pain   The mechanism by which the muscles of the anterior abdominal wall
become tender in disease of the abdominal viscera is unknown and its
discussion irrelevant to our main theme   But it seems clear that the dis-
turbance of the muscles is cut short by paralysing temporarily their motor
and sensory supply, and that it usually takes time to be re-established

In some of these observations, pain present initially disappeared during
anæsthetisation of the abdominal wall, even though gastric acidity remained
high   We would emphasise that the point as to whether pain can or cannot
be elicited from the stomach when the anterior abdominal wall is anæsthetic
is too important to be decided by observations designed to settle an entirely
different issue   We therefore refrain from drawing any conclusions until
properly designed experiments have been made

Had we been able to demonstrate tenderness localised to the ulcer
crater, and shifting with it, when the anterior abdominal wall had been
anæsthetised we should have had evidence concerning the site of origin of
ulcer pain   Our failure to do this, and the conclusion that tenderness lies
chiefly in the anterior abdominal wall leaves the position once more open
and it may now be attacked from a different approach

The mesentery and posterior abdominal wall considered

The difficulty which lies in the way of the simple hypothesis that pain
in peptic ulcer is due to stimulation of pain nerve endings by acid arises from
the doubt as to whether such nerve endings actually exist in the stomach and
duodenum , it is a difficulty that is not peculiar to the chemical hypothesis,
applying equally to the tension hypothesis, and in no way weakens the
conclusions reached in the previous paper   Because of this difficulty it is
relevant to enquire into the alternative   Lennander (9) found that all the
hollow viscera and their mesenteries were insensitive to direct stimulation,
but that the parietal peritoneal aspect of both anterior and posterior
abdominal walls was extremely sensitive   He therefore supposed (10) that
the abdominal pain of visceral disorder was due to stimulation of pain nerve
endings in the abdominal wall either by stretching them, as for example
through traction on the base of the mesentery, or by chemical irritation
consequent on lymphatic spread of inflammatory products from the diseased
viscus   In the case of peptic ulcer, he supposed that pain arose from an
infective lymphangitis around the coeliac artery, the aorta and œsophagus,
both halves of the diaphragm and the anterior and posterior mediastinum,
with consequent enhanced irritability of the neighbouring pain nerve endings
The attacks of pain were thought to arise from irritation of these nerve
endings by the arrival thereabouts of lymph highly charged with HCl, and
epigastric tenderness to the enhanced irritability towards mechanical
stimulation of these nerves

There is clear evidence from the skin that lymphatic spread of active
substances may occur   Thus the injection of adrenaline subcutaneously
may be followed by the appearance of blanched streaks running proximally

along the course of the veins from the site of injection   Some years ago one of us (G W P) observed in his wife that a burn of the skin over the knuckle of the index finger was followed in about 2 hours by intense red streaks about 2 mm wide close to the plexus of veins on the dorsum of the hand, and following these proximally to the wrist , there was no abrasion of the skin and no infection   A similar phenomenon after freezing and ultraviolet irradiation of the skin was observed by Lewis and Zotterman (16) Lewis and Hess (14) have also attributed the spread along the course of the veins of tenderness after local injury of the skin to diffusion of injury products through the lymphatics   In the last two instances we see substances released from the skin by injury producing their effects at a distance by conduction by, and diffusion from, lymphatics

In the particular case of peptic ulcer there is evidence of the diffusion of injury products from the affected viscus into lymphatics for it is a frequent experience at operation in peptic ulcer to find the lymphatic glands on the greater and lesser curvatures of the stomach enlarged and pink   Glands obtained by us at operation from two cases of active gastric ulcer showed conspicuous hyperæmia in both and œdema in one   Professor C A Pannett has also informed us that a very frequent operation finding is inflammatory thickening of the lesser omentum   There is therefore a prima facie case for the hypothesis that pain in peptic ulcer arises from pain nerve endings at a distance from the viscera through the lymphatic spread of injury products from the ulcer or its neighbourhood   Lennander's hypothesis, implying as it does a chemical stimulation of pain nerve-endings, is in general conformity with the conclusions reached in the previous paper   The chief difficulty in accepting it is the time relations of the pain in response to the application and withdrawal of the chemical stimulus, acid   These time relations and their implications will now be considered

The time relations of gastric ulcer pain

In the previous paper it was mentioned that introduction of acid into the stomach does not at once give rise to pain , usually it does so only after a latent period of several minutes   Similarly a latent period elapses between emptying the stomach or administration of alkali and the complete abolition of pain   These time relations for ulcer of the stomach are summarised in Table II , the data for duodenal ulcer are similar, but since the activity of the pylorus introduces an additional unknown factor we have not included them in the Table   The first column shows the interval elapsing from the beginning of acid injection to the onset of pain , 200 to 300 c c of $\frac{N}{20}$ to $\frac{N}{10}$ acid was injected in 1 to 3 min , pain began 3 to 19 minutes after beginning the injection   The second and third columns give the data for emptying the stomach, the second column giving the duration of aspiration and the third the time from the beginning of aspiration to the end of pain   In each case pain began to lessen during the aspiration , in one case it ended before the stomach was fully emptied, and in the remainder 0 to more than 14

TABLE II

Time relations of pain produced in patients with gastric ulcer by $\frac{N}{20}$ to $\frac{N}{10}$ HCl and relieved by aspiration and administration of alkali

PATIENT	From beginning acid injection to pain	From beginning aspiration to		From giving alkali to	
		end of aspiration	end of pain	pain easing	pain gone
R C F	9	—	—	—	4
P H	7 18	2 15	3 20	— 2	— 0
R N	12	3	8	—	—
W G	18 3	2 4	13 18*	— —	— —
F C	5 19	1 3	4 4	— —	— —
J N	6 5 16 19	15 — 10 3	14 — 10 4	— 3 — —	— 8 — —
L C	6	—	—	—	—
B	Natural pain	—	—	2	17
A E	Natural pain Natural pain	— —	— —	$\frac{3}{4}$ $1\frac{1}{2}$	4 5
J S *	9 25 Natural pain	— — —	— — —	— 1 5	23 19 32
R W †	Natural pain	—	—	1	3
H H *	17	—	—	—	—

* Carcinomatous ulcer of body of stomach

† Anastomotic ulcer verified at operation

minutes later   The last two columns give the times for relief of pain after introducing 4 g of a mixture of sodium magnesium calcium and bismuth carbonates suspended in 30 c c water into the stomach   in the cases where pain had been produced by acid, the alkali was injected through the stomach tube, and in the instances with natural pain, it was swallowed, the operation in all cases taking not more than 15 sec   Data are also included for two cases of carcinomatous ulcer in which the latent periods were rather strikingly long, and for one case of anastomotic ulcer in which a large ulcer on the anastomosis between stomach and jejunum, and floored by liver, was excised two weeks later

When acid is injected into the empty stomach of a patient with a gastric ulcer, we may assume that acid comes into contact with the ulcer not earlier than the beginning and not later than the end of injection   The interval which elapses between contact of acid with ulcer and onset of pain may therefore be, in the instances given, between 2 and 19 mins , and averages between 9 and 11 minutes according as to whether pain is timed from the beginning or end of injection   Ten minutes may thus be taken as a representative latent period   This period presumably represents the time elapsing from contact of the injurious agent, acid, with the vulnerable area of the stomach wall, until the chemical excitant has accumulated around the pain nerve endings to an adequate or threshold level

The time relations for the relief of pain are less easy to analyse   Thus aspiration of the stomach in many instances occupied a substantial part of the time taken for the disappearance of pain, and there is no certainty that the stomach was completely emptied in all cases   The exact times elapsing between withdrawal of acid from contact with the vulnerable area and the lessening and end of pain are thus more difficult to estimate, but in one observation on P H , two on F C and one on J N the times to the end of pain must have been less than 4 minutes   The introduction of alkali into the stomach was accomplished quickly, but here again the period necessary for mixing of the stomach contents and for reaction of the largely insoluble carbonates with HCl are unknown , the degree of neutralisation is also unknown   Nevertheless, pain was observed to lessen in $\frac{3}{4}$ to 3 minutes and to end 4 to 17 minutes after the administration of alkali   Averaging the times for relief of pain by alkali in the 5 observations on gastric ulcer we obtain round figures of 2 minutes for decline and 8 minutes for abolition of pain   Thus it appears that when the injurious agent acid is withdrawn from the vulnerable area of the stomach wall, the concentration of the excitant substances around the pain nerve endings has declined appreciably in about 2 minutes and has fallen below the threshold of pain in about 8 minutes

To interpret these time relations in terms of the spatial relationship between the inner surface of the stomach wall and the pain nerve endings, we have sought guidance from the time relations obtained from ulcers of the skin, where the location of the nerve-endings is known

The time relations of skin ulcer pain

In three normal male subjects the superficial layers of the skin of the forearm were shaved off through the papillæ of the corium over an area about 1 cm square   To test these ulcers, they were surrounded by a rampart of plasticine cemented to the intact surrounding skin and warm isotonic solutions of 0 85% NaCl, 0 15 N HCl and NaOH were injected into the cup so formed from a pipette   The subject was unaware what solutions were being applied and his only task was to announce any change in his sensations from the areas concerned   The results are summarised in Table III   The first experiments were made on the day following the preparation of the ulcer, when spontaneous pain had subsided and the surface of the ulcer was

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TABLE III

Showing the time relations of pain produced from a skin ulcer by application of 0 15 N HCl and relieved by neutralisation with 0 15 N NaOh

State of skin ulcer	No of observa tions	ACID						NEUTRAL			
		Pain begins		Pain maximum		Duration acid		Pain lessens		Pain gone	
		min	sec	min	sec	min	sec	min	sec	min	sec
1 Subject G P											
Ulcer 16 hr plus mucus	2	0	8	0	47	1	22	0	12	0	51
Ulcer 96 hr Scab on	3	0	35	2	1	3	24	0	47	1	36
	2	5	7	8	30	18	0	0	2	0	15
Scab off	1	0	25	3	30	10	0	0	20	6	0
2 Subject G B											
Ulcer 20 hr plus mucus	3	0	5	0	47	2	28	0	5	0	40
Ulcer 72 hr Scab on	2	0	39	0	52	2	33	—		1	0
	1	2	47	8	7	9	30	0	43	3	48
Scab off	1	0	4	0	53	10	57	1	2	2	56
3 Subject G B											
Ulcer 16 hr plus mucus	3	0	14	1	0	2	2	0	15	2	3
Ulcer 96 hr Scab on	2	0	45	2	18	3	18	0	58	1	47
	1	3	20	6	15	11	0	1	20	5	20
Scab off	1	0	15	3	0	0	14	0	45	2	45
4 Subject M H											
Ulcer 20 hr plus mucus	3	0	13	0	36	2	15	0	6	0	46
Ulcer 72 hr Scab on	2	0	15	1	11	2	18	0	22	2	0
	1	6	3	9	48	13	8	0	47	3	20
Scab off	1	0	2	2	10	13	28	0	5	2	15
5 Subject G P											
Ulcer 20 hr Ulcer 110 hr Scab on	3	0	7	0	55	2	20	0	16	1	28
Scab off	1	3	15	9	20	2	40	1	55	5	55
	1	0	28	5	58	10	3	0	25	8	55

moist with serous exudate This exudate was absorbed on gauze before testing The application of isotonic sodium chloride to such ulcers was in all subjects painless 0 15 N HCl produced pain in 15 seconds or less reaching a maximum in 1 minute in all 5 experiments Neutralisation of acid by addition to the cup of an equal volume of 0 15 N NaOH led to decline of pain in 16 seconds or less and to its end in a little over 2 minutes or less in all the observations These times are all much shorter than the corresponding times for ulcer of the stomach, but the ulcers themselves are rather different in construction Thus the base of a peptic ulcer always or nearly always consists of an avascular layer of necrotic material Moreover the surface of the stomach is normally protected by a layer of mucus and it is possible that in certain instances this may extend over the ulcer

The effect of covering the ulcer with a thin layer of mucus is shown in the second observation of experiments 1 to 4 Mucus was obtained from the subjects own spit, and concentrated by evaporation in a watch glass at 37° until it was tenacious The ulcer was then covered with it, and after about 10 minutes to allow a firm adherence, the tests were made In each of the 4 experiments the times taken for pain to develop with acid and to decline with neutralisation were lengthened, though the effects were slight

After 3 or 4 days these skin ulcers were covered with a scab formed by the drying and hardening of the serous exudate The effects of the scab on the responses of the skin ulcers are shown in the last 2 observations in each of the 5 experiments, recording the responses with the scab in place and after it had been pulled off to leave a raw surface exuding serum With the scab in place, pain did not develop until between 2¾ and more than 6 minutes, and continued to increase until between 6¾ and nearly 10 minutes after acid was first applied Similarly when acid was neutralised pain did not begin to decline until between 43 seconds and 2 minutes , and pain did not disappear until between 3 minutes 20 seconds and 15 minutes In these experiments with the scab, acid was left in contact with the ulcer much longer than it had been in the previous observations and in the control observation after the scab had been removed acid was left on for comparable times It will be seen that removal of the scab reduced conspicuously the interval between application of acid and the appearance and levelling off of pain The times for decline of pain after neutralisation were also reduced conspicuously in four of the five experiments, and in the exceptional case (Experiment 2) the ulcer was exuding serum very rapidly The times for disappearance of pain were also reduced, in 4 of the 5 experiments, but all these times were significantly longer than had been obtained with the fresh ulcer to which acid had been applied for a much shorter time

In the observations where acid was applied to the skin ulcer protected by scab, itching of the skin was commonly experienced either a little before or during pain In one subject with a fair skin a definite flare developed around the ulcer These phenomena, which Lewis (11) has previously observed, indicate that acid injures the cells of the skin with consequent

liberation of a histamine like substance   The possibility is thus raised that
it may not be the hydrogen ion itself but some substance released by cellular
injury which is the actual excitant to the pain nerve-endings   This suggestion
is further supported by the very long persistence of pain after neutralisation
of acid in many of these experiments   On the other hand the very short
latent period, 2 seconds, in Experiment 4, and 4 seconds in Experiment 2,
between the application of acid and the appearance of pain with unprotected
ulcers indicates that the hydrogen ion itself may also stimulate pain nerve
endings   On the evidence therefore it may be concluded that when acid is
applied to an ulcer of the skin pain nerve endings are stimulated in part by
the excitant action of hydrogen ions, and possibly in part by the liberation
of another excitant substance from injured cells

   The conspicuous lengthening of the latent periods, produced in ulcer
of the skin by the presence of a scab is not difficult to understand   For
whether or not the actual excitant of the pain nerves is the hydrogen ion,
the ultimate cause of pain is the attainment of an adequate level of hydrogen
ion concentration at some point in the living tissue   When acid is applied
to an unprotected ulcer this must happen quickly, and neutralisation may be
assumed to arrest the accumulation of hydrogen ions in the surface layers
equally quickly, though it may take longer for the deeper layers to regain
their usual reaction   A scab offers a barrier through which diffusion must
occur before change on the surface of the ulcer can be reflected in a
corresponding change in the surface layers of living tissue, and this delay
will occur whether the change on the surface be from neutral to acid or from
acid to neutral   Further it may be surmised that since the tissues are
capable of removing excess hydrogen ions, being supplied with blood and
buffered at a slightly alkaline reaction, the delay will be greater when the
change on the surface is from neutral to acid than when it is from acid to
neutral   These expectations are fulfilled by the changes observed in the
time relations of the pain

   As far as time relations of pain are concerned an unprotected ulcer of
the skin thus behaves quite differently from ulcer of the stomach when the
reaction at the surface is changed from neutral to acid or from acid to neutral
On the other hand the time relations for stomach ulcer are very similar
to those for an ulcer of the skin protected by scab   Thus considering first
the latent period between application of acid and onset of pain, the data
are as follows   The limits for 12 observations on ulcer of the stomach were
2 and 19 minutes, and the average 10 minutes   The limits for 5 observations
on ulcer of the skin covered by scab were 2 minutes 47 seconds and 6 minutes
3 seconds and the average 4 minutes 8 seconds   Considering again the latent
period between the neutralisation of acid and the lessening of pain, the data
are as follows   The limits in 5 observations on gastric ulcer were 45 seconds
and 3 minutes, and the average 2 minutes   The limits for 5 observations on
skin ulcer covered by scab were 43 seconds and 2 minutes, and the average
1 minute 21 seconds   Finally the interval between neutralisation and

abolition of pain was as follows   The limits for 5 observations on gastric ulcer were 4 minutes and 17 minutes and the average 8 minutes   The limits for 5 observations on skin ulcer covered by scab were 3 minutes 20 seconds and 15 minutes and the average 6 minutes 41 seconds   While it is clear that on the average the times are shorter for skin ulcer covered by scab than for gastric ulcer, they are of the same order in the two instances and overlap to a considerable extent

The thickness of the scab covering the skin ulcer was measured in Experiment 1 of Table III and found to 0 29 mm   We could not examine histologically the gastric ulcer at the time of observation   But in three other gastric ulcers examined after surgical resection and subsequent fixation and microscopic section and staining, the thickness of the layer of necrotic material forming the floor of the ulcer was found to average respectively 0 24, 0 36 and 0 49 mm

We have now compared the time relations of pain produced by the same stimulus, acid, in two kinds of ulcer   in skin where the location of the pain nerves is known, and in the stomach where it is unknown   In the skin the pain nerves are almost certainly excited chemically   In the stomach all the evidence is in favour of a similar hypothesis   It is clear that the time relations of peptic ulcer pain are out of line with the idea that the pain nerve endings lie exposed on the surface of the ulcer , they are much too long   The time relations are however entirely consistent with the hypothesis that the pain nerves are situated in the floor or sides of the ulcer and separated from the cavity of the stomach by a layer of, from this point of view, functionally inert material   In extending ulcers, the slough covering the ulcer base would do for this layer, since it is of the expected order of thickness   On this hypothesis the pain fibres might arise from many structures such as omentum, liver, pancreas and posterior abdominal wall which in large chronic ulcers may contribute to the ulcer crater   But in acute ulcers (for example R C F , Table II), the implication is of nerve endings lying in the wall of the stomach itself

In answering the question as to whether the time relations observed for pain in gastric ulcer are also consistent with Lennander's hypothesis, the chief difficulty is the lack of a yardstick, for we know of no comparable instance where pain is due solely to stimulation of pain nerves lying at a distance from the site of injury through lymphatic spread of excitant substances   We are thus limited to a theoretical discussion   Lennander supposed that the actual excitant to the pain endings might be acid   In view of the buffering power of the tissues, of blood and of lymph we find it inconceivable that excess hydrogen ions absorbed at the site of the ulcer can travel in the lymphatics through the mesentery, there mixing with other lymph, and diffuse out to the neighbourhood of the nerve endings of the posterior abdominal wall in quantities sufficient to have an excitant action   The only conceivable mechanism would be the release of a more stable substance from the site of injury by acid, namely the ulcer crater, and its

subsequent lymphatic transport In terms of this hypothesis the latent period between the application of acid and the onset of pain would represent the time taken for release of an adequate amount of the excitant at the site of injury in the stomach wall and its entry into, carriage by, and diffusion out of, the lymphatics The work of Drinker and Field (5) has shown that lymphatic transport may be extremely quick , dye injected subcutaneously in the foot of a dog may appear in the thoracic duct in 10 seconds. Ten minutes, the average latent period for the development of pain would seem therefore to allow ample time for these several processes inherent in Lennander's hypothesis It is not so however with the relief of pain by alkali For as we have seen pain may lessen in under a minute and disappear in 4 minutes In many known instances where substances are released from cells by injury, this release continues for some time after the injurious agent is withdrawn We cannot believe that the concentration of the excitant which is, ex hypothesi, relatively stable and which is formed at the site of injury and transported by the lymphatics for many centimetres can fall around the pain nerve endings of the posterior abdominal wall in as short a time as 1 minute after the withdrawal of acid from contact with the stomach wall

We have experienced a further difficulty in accepting Lennander's hypothesis Gray (6) has shown that the lymphatics of the stomach consist of an extremely fine plexus of freely anastomosing channels. Lymph that finds its way via a single channel to the posterior abdominal wall and thence to the thoracic duct, comes not from one area of the stomach but from many By the time the posterior abdominal wall is reached lymph coming from the ulcerated areas must be much diluted with lymph from more normal regions and the concentration of pain excitants correspondingly reduced

Unusual reference of pain

Three of our cases complained of pain localised to the iliac fossa, on the left in 2, on the right in 1, with some radiation to the back at the same level , two of them were anaemic consequent on gastrointestinal haemorrhage and presented some initial difficulty in diagnosis In each case the pain was relieved by vomiting and alkali and, in the only two where the experiment was made, was reproduced by acid These three cases all had large penetrating ulcers on the posterior aspect of the lesser curvature of the stomach, measuring on the X-ray film 3½, 3½ and 2½ cm in diameter and 2½, 3 and 1 cm in depth Two healed slowly on medical treatment, one was completely refractory and at operation was shown to have invaded the pancreas which formed its floor

A fourth case after partial gastrectomy developed pain in the left anterior axillary line with cutaneous tenderness over the same area which covered the 7th and 8th ribs This pain was not abolished easily by morphine but was abolished by hot milk A tumour later presented in the left hypochon-

drium under the tender area and at operation an anastomotic ulcer adherent to the anterior abdominal wall, was excised

The unusual reference in these cases of pain, having otherwise the properties common to that of ulcer, clearly indicates that pain arose from an unusual site If in the usual case of gastric ulcer pain arises from the stomach or its mesenteric attachments, in these cases a different source must be found, and the operation and X-ray findings suggest the pancreas in the first three and the anterior abdominal wall in the fourth , for it was these tissues which were exposed in the floor of the ulcer to the varying acidity of the gastric content   Of the two cases in which pain was reproduced by acid one (F C) with pain in the left iliac fossa is included in Table IV   In the other (F H) pain in the right iliac fossa began 20 minutes after introducing $\frac{N}{10}$ acid into the stomach and began to ease 1 minute after injecting 4 g of $NaHCO_3$ in 40 c c water into the stomach   These times are similar to those encountered in other cases with small ulcers supporting the conclusion that the ulcer crater is the site of the pain nerve endings

Discussion

The chief facts relevant to the location of the nerve endings concerned with pain in gastric ulcer lead to conflicting conclusions   On the one hand direct tests at surgical operation suggest that the stomach is insensitive and that the nearest pain sensitive tissue is the posterior abdominal wall , the inference here is Lennander's hypothesis of lymphatic spread of pain excitants from the ulcer to the posterior abdominal wall   On the other hand a detailed study of the time relations of the pain is not easily reconciled with Lennander's hypothesis but reveals close conformity with the behaviour of an ulcer in which pain nerve endings are known to lie but a small distance beneath the surface , the inference here is that the pain nerve endings lie in the ulcer crater, and thus in acute ulcers at least, in the stomach wall

It is of some importance that a similar conflict of evidence exists regarding pain from the only other viscus studied in comparable detail, the heart   The very few records of operation on the conscious subject agree (1) that the heart can be pinched and pricked without the subject feeling pain , a hypothesis was at one time widely supported that anginal pain arose not from the heart but from the aorta   On the other hand a detailed study of the behaviour of anginal pain in the intact subject leaves little doubt that its source lies in the heart muscle itself (13, 27)

In seeking to resolve this dilemma it should be pointed out that the contradictory conclusions concerning the source of pain in gastric ulcer have been drawn from observations made under entirely different circumstances In the one the subject has been subjected to the procedures' inherent in opening the abdomen before the observations were made , in the other no such disturbances have occurred   We are thus led to enquire as to the possible

fallacies in the conclusion drawn from surgical operation namely that the stomach wall is devoid of pain nerves   The chief possibilities are as follows

1   The mental state of the subject who has had his belly opened is scarcely normal   No one who has made experimental observations on pain in human subjects can fail to be aware of how the sensation of pain may be lessened or abolished by strong sensory stimuli from elsewhere which compete for the subject's attention   In the case of ulcer we have repeatedly observed that the mere swallowing of a Ryle's tube may lead to a transitory disappearance of pain which returns after a few minutes

2   In many instances the subject has received morphine or a period of general anæsthesia before the observations have been made

While it would be wrong to suppose that these two possibilities should be dismissed entirely it is doubtful whether they can alone explain the absence of pain when the stomach is disturbed, for in many of these subjects pain has been elicited from traction on the stomach and interference with the anterior abdominal wall

3   Anæsthetisation of the body wall may interfere with the capacity to elicit pain from an underlying viscus   It has been shown here that most of the tenderness associated with peptic ulcer is located in the anterior abdominal wall and most probably in the rectus abdominis muscle   It is not impossible that pain may arise from sustained contraction of muscle fibres excited reflexly from the gastric or duodenal ulcer   Although in several instances ulcer pain was observed to disappear during anæsthetisation of the belly wall, we deliberately refrain from drawing any conclusion because we have not yet made observations specifically designed and properly controlled to test this hypothesis

4   Hurst suggested that the pain nerves in the stomach would respond only to a certain specific stimulus which he supposed to be tension (7)   As Lewis and Kellgren (15) have pointed out this suggestion cannot be allowed because all nerves, even the specialised optic nerve, respond to section   Any pain nerves in its wall must be stimulated when the stomach is cut through

5   The stimuli applied to the exposed stomach at surgical operation may not be adequate in duration, for it is possible that some degree of temporal summation must occur in the central nervous system before pain is experienced as a result of impulses arising from pain nerves in the stomach   Such a hypothesis would explain the apparent conflict of evidence   For cutting, pinching or pricking the stomach would give rise to momentary discharge from the nerve fibres concerned, while the long continued presence of a chemical irritant such as acid would lead to a more prolonged discharge of nervous impulses   There is however as yet no direct evidence

6   In both the heart and the stomach, pain nerves may be relatively few and confined to certain parts   It is known for example that acute peptic ulcers may be painless until they perforate into the peritoneal cavity, likewise myocardial infarction may occur without pain, examples of both have occurred in our own experience   Most stomach ulcers are on the posterior wall and on the lesser curvature of the stomach, and it may be that these are the areas of stomach which contain most pain nerves, and that the parts nearer the greater curvature and on the anterior wall which have been most intensively explored at operation contain least   It would not be difficult to assemble information bearing on this point, but to our knowledge this has not yet been done

This brief review indicates that there are a number of possible fallacies as yet imperfectly explored in the conclusion from operation findings alone that the stomach is devoid of pain nerves   We conclude therefore that there are not sufficient grounds for rejecting the hypothesis developed in this paper

The hypothesis that ulcer pain arises from chemical stimulation of nerve endings lying in the crater but separated from the contents of the gut by a functionally inert layer is in accord with certain other facts hitherto unexplained   Thus we have pointed out that sensitivity to pain produced by injection of acid into the stomach may be lost when an ulcer crater is still demonstrable   Now it would accord with all that is known of repair elsewhere if nerve fibres did not at once grow into the granulation tissue which steadily fills up the crater   A stage will come therefore when a crater is still present but when the nerve free layer is thick enough to protect the underlying endings from changes in hydrogen ion concentration previously adequate to excite   Again it has been observed repeatedly that ulcer pain may be abolished following a hæmatemesis   As Schindler (26) has shown by gastroscopy, the ulcer crater following hæmorrhage may be filled by blood In terms of the present hypothesis this blood would again increase the thickness of the protective layer over the pain nerve endings and thus prevent their excitation by changes in acidity in the cavity of the stomach

Summary

1   The observation made by Lennander and others has been confirmed in one case namely that after the anæsthetised belly wall has been incised no pain is experienced when the stomach is cut or pinched   The possible fallacies in concluding from such an observation that pain cannot be elicited from the stomach are discussed

2   When the anterior abdominal wall is anæsthetised by injecting novocaine around the appropriate intercostal nerves, tenderness is, in most patients with peptic ulcer, completely abolished   It is concluded that most

if not all of the tenderness associated with peptic ulcer is located in the anterior abdominal wall and most probably in the rectus abdominis muscle

3 When acid is injected into the stomach of a patient with a gastric ulcer, pain begins after a latent period of about 10 minutes When acid is neutralised by alkali, pain lessens after about 2 minutes and disappears after about 8 minutes

4 The time relations for the onset of pain when acid is applied to a fresh skin ulcer, and for its relief when the acid is neutralised are very much shorter The times are slightly prolonged by covering the ulcer with a thin film of mucus When however the skin ulcer is protected by a scab, the time relations of the production of pain by acid and its relief by alkali are greatly prolonged, and are then of the same order as observed in ulcer of the stomach

5 The time relations of pain in peptic ulcer are compatible with the chemical stimulation of pain nerve endings situated in the ulcer crater and separated from the cavity of the stomach by a layer of, from this point of view, functionally inert material

6 Because ulcer pain is relatively quickly relieved by alkali and for other reasons we reject Lennander's hypothesis that pain arises by stimulation of nerve endings in the posterior abdominal wall by excitants carried from the ulcer area through the lymphatics

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THE USE OF A PORTAL ANASTOMOTIC VEIN FOR ABSORPTION STUDIES IN MAN

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A PATIENT suffering from hepatic cirrhosis was seen in whom a large anterior abdominal wall vein was believed to communicate with the portal venous system   Observations were made on the contents of this vein during intestinal absorption   Although the studies were brought to a premature conclusion by thrombosis of the vein, the results are reported in the hope that they may stimulate interest in the possible research value of such veins in similar patients

CASE REPORT

F.M., an old age pensioner aged 71 was admitted to hospital on 8 11 45

History   For several years there had been recurrent epistaxes , now occurring nearly every week and initiated by blowing the nose   Appetite was fair and most foods were well tolerated   The bowels were constipated and the motions occasionally streaked with blood   The early morning urine was sometimes rather dark in colour   There was morning cough with white sputum   The patient thought he was losing weight, the exact amount being uncertain   Past health had been good and he had never suffered from jaundice   He drank 1 pint of beer daily, and lived alone in a single room   his diet being mainly pies and breakfast sausage   He did not eat his meat, bacon or butter rations and rarely took vegetables

Examination showed a thin old man   ' Spider " angiomata suggestive of liver disease were seen on the cheeks   Dilated vessels on the nasal septum were a possible source of the epistaxis   The chest showed a few dry rhonchi but no moist sounds   The abdomen was not distended and shifting dullness could not be elicited   A large dilated tortuous superficial abdominal vein in the right lower quadrant (Fig 1) was non pulsatile and there was no murmur over it   On emptying it rapidly filled from above downwards   Superiorly the vein emerged about 3 cm above the umbilicus, apparently through a paraumbilical hernia   Inferiorly it joined the great saphenous vein just below the inguinal ligament   The liver edge was firm and easily palpable 4 cm below the right costal margin in the nipple line   The spleen could not be felt   There was no peripheral oedema   Rectal examination showed internal hæmorrhoids   *Urine*   S G 1015, contained no albumin or bile pigments   Urobilinogen was present in an early morning specimen

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Investigations Serum bilirubin 0 9 mg per 100 c c , serum alkaline phosphatase 16 units per 100 c c , serum cholesterol 265 mg per 100 c c , serum proteins 6 2 g per 100 c c with 3 5 g albumin, 2 7 g globulin, and A/G ratio 1 3 Serum colloidal gold reaction (18) negative Oral hippuric acid synthesis test (20), 2 5 g (as sodium benzoate) excreted in 4 hours *Blood count* Hb 10 2 g per 100 c c (65% Haden normal), R B C 4,600,000 per c mm , W B C 7,000 per c mm Wassermann reaction negative *Barium meal* examination showed no gastrointestinal abnormality

Aspiration liver biopsy (21) sections show a hepatic cirrhosis Surviving liver cells, apart from some fatty change at the periphery of the nodules, contain their usual complement of glycogen and appear healthy

As this anastomotic vein probably communicated with the portal venous system, it was considered that its blood might be draining the alimentary tract The abdominal vein was thin walled and tortuous which made sampling difficult This difficulty and the consequent discomfort to the patient limited the frequency of sampling Arm veins also were small and difficult to needle

Preliminary metabolic considerations

Weight of patient 60 kg Height 148 cm

During the period of study the patient consumed a weighed diet of 2,175 calories daily , 359 g carbohydrate, 133 g protein and 23 g fat 30 g brewer's yeast daily was included

Over two three-day periods on this regime the daily output of urinary nitrogen averaged 9 3 g , the faecal nitrogen 1 5 g , and the faecal fat 7 2 g The patient thus showed a positive nitrogen balance, and on this diet the faecal fat was within normal limits During his stay in hospital he gained 8 kg in weight All the observations were made in the morning, the last meal being at 8 p m the previous night

Carbohydrate tolerence tests

Galactose 40 g galactose in 50 per cent solution was given orally and one hour later samples were taken from the abdominal wall vein and a right antecubital vein

The galactose content of the abdominal vein was 20 mg per 100 c c , and that of the arm vein 7 mg , per 100 c c

Lævulose and Glucose To facilitate more frequent sampling drip infusions of heparinized normal saline were set up in both the abdominal and an antecubital vein Fasting venous samples were removed from both sites, and then 50 g lævulose and 50 g glucose were given by mouth Further venous samples were taken 30, 60 and 90 minutes later

From Table I it is seen that —

1 In the fasting state the glucose concentrations in the two veins is the same No lævulose can be detected in either In later experiments a fasting sample was not taken from the abdominal vein its composition was assumed to be identical with that of the systemic

2 During the 90 minutes after the sugar was given, the glucose and lævulose content of the abdominal vein is conspicuously greater than that of the antecubital vein

3 The highest value for glucose in the abdominal vein occurred at 30 minutes, that for lævulose at 60 minutes

4 During the absorption period, in neither vein was there any significant change in the serum potassium or serum ester phosphate concentrations

TABLE I

Biochemical changes in the abdominal wall vein and the antecubital vein after oral glucose and lævulose

Time after sugar minutes	Concentrations in abdominal wall vein				Concentrations in antecubital vein			
	Glucose mg/ 100 c c	Lævulose mg/ 100 c c	Ester Phosphate (as P) mg/ 100 c c	Potas sium mg/ 100 c c	Glucose mg/ 100 c c	Lævulose mg/ 100 c c	Ester Phosphate (as P) mg/ 100 c c	Potas sium mg/ 100 c c
0	112	0	0 6	21	112	0	0 6	19
30	230	25	0 5	20	182	8 4	0 5	—
60	123	84	0 7	22	84	24 6	0 5	20
90	159	24	0 7	21	132	6 6	0 2	21

Fat tolerance tests

These were done by the method of Dixon, Drew and Samuel (8)   The meal consisted of 75 g dairy butter prepared with a barium sulphate suspension   After a fasting blood sample had been taken from an antecubital vein, a duodenal tube with mercury tip was passed into the second part of the duodenum and its position confirmed by X-ray screening   The meal was given down the tube and seen to pass through the duodenum and enter the upper coils of jejunum   Venous samples were taken from the abdominal and antecubital veins two and three hours after the meal

From Table II it is seen that no significant change in serum opalascence, lipid phosphorus, cholesterol, total fatty acid, non-phospholipid fatty acid or neutral fat concentrations could be demonstrated   Results for a typical normal control subject are also shown

This result was most unexpected and the observation was therefore repeated   This time 50 g glucose was included in the meal, and its rate of progress was followed radiologically at intervals for 2 hours   Although the rate of transit of the meal through the intestines was normal, and although a glucose concentration increase was shown in both veins, that in the

TABLE II

Biochemical changes in the abdominal wall and antecubital veins after a fatty meal with and without added lipase

Minutes after fatty meal	Abdominal wall vein						Antecubital vein					
	Lipid phosphorus mg/100 cc	Cholesterol mg/100 cc	Total fatty Acid MEQ/1,000 cc	Non-phospholipid Acid MEQ/1,000 cc	Neutral fat mg/100 cc	Serum opalescence	Lipid phosphorus mg/100 cc	Cholesterol mg/100 cc	Total fatty Acid MEQ/1,000 cc	Non phospholipid fatty acid MEQ/100 cc	Neutral fat mg/100 cc	Serum opalescence
Fatty meal alone												
0	—	—	—	—	—	—	7 7	225	12 8	8 7	260	0
120	7 9	190	11 8	7 6	230	0	7 7	171	11 8	7 6	230	0
180	8 2	212	12 1	7 7	230	0	8 0	183	12 1	7 9	240	0
Fatty meal + lipase												
0	—	—	—	—	—	—	4 8	101	12 6	8 6	250	0
60	5 4	102	12 6	8 1	240	0	5 3	104	12 2	7 8	230	0
120	5 3	96	13 3	8 9	270	+	5 4	95	12 2	7 8	230	0
Normal control subject												
0	—	—	—	—	—	—	8 5	209	8 5	4 5	130	0
120	—	—	—	—	—	—	10 6	224	10 6	6 2	180	+
180	—	—	—	—	—	—	14 9	211	14 9	10 4	310	++

TABLE III

Biochemical changes in the abdominal wall and antecubital veins after a protein and carbohydrate meal

Minutes after meal	Concentrations in abdominal wall vein			Concentrations in antecubital vein		
	Glucose mg/100 cc	Non protein Nitrogen mg/100 cc	Urea Nitrogen mg/100 cc	Glucose mg/100 cc	Non protein Nitrogen mg/100 cc	Urea Nitrogen mg/100 cc
0	—	—	—	94	31	14
120	119	66	15	72	46	15

abdominal vein being greater than that in the antecubital, there was still no change in the fatty substances   It was decided to repeat the experiment a third time and to give a lipase preparation with the butter fat

The lipase was made from fresh pig's pancreas (5) and its potency tested 100 c c of this extract was mixed with the fat meal and given under X-ray control into the second part of the duodenum   Venous samples were taken from an arm vein before the meal and from both arm and abdominal veins 1 and 2 hours afterwards

From Table II it is seen that in the abdominal vein 2 hours after the fatty meal there is a slight serum opalescence and an increase in total fatty acid, non-phospholipid fatty acid and neutral fat concentrations   The concentrations of phospholipid and cholesterol show no change   During the 2-hour period there has been no detectable systemic lipæmia

Protein tolerance test

A fasting sample was withdrawn from an antecubital vein and the patient then ate a meal consisting of meat, cabbage, potatoes, bread and concentrated skimmed milk   It was equivalent to 86 g carbohydrate, 62 g protein and 6 g fat   Two hours later samples were withdrawn from the abdominal vein and from the antecubital vein

From Table III it is seen that (1) 2 hours after the meal there is a significant difference between the glucose concentrations of the two veins , (2) the urea nitrogen concentration has remained relatively constant in both veins , (3) 2 hours after the meal the non-protein nitrogen concentration has increased in both veins, but more conspicuously in the abdominal vein

Venograms of the abdominal vein

Blood flow in the vein was stopped by digital pressure   A needle was introduced above and close to the 'point of occlusion and 20 c c of a radio-opaque solution (Pyelosil) injected   The injection was therefore retrograde X-rays of the right upper quadrant of the abdomen were taken (Fig 2) The vein starts as a very small tributary passing down over the costal margin (D)   It is joined (C) by a larger vein which comes from above and to the right   C represents the point where this deep vein penetrates to the subcutaneous tissue   As the vein proceeds downwards towards the inguinal ligament it becomes very tortuous (A)   The surface marking of the umbilicus is also shown (B)   It was hoped to take another venogram using a larger volume of pyelosil, but unfortunately the first injection was followed by complete thrombosis of the vein, although pyelosil is usually considered non-irritant to vascular endothelium

A superficial portion of vein 10 cm long contained about 12 c c, of " pyelosil " This portion when emptied of blood filled again in 2 seconds The blood flow through the abdominal vein was therefore believed to be between 300 and 400 c c per minute

Discussion

The biochemical results and the venogram make it almost certain that the anterior abdominal wall vein communicated with the portal venous system In hepatic cirrhosis the intrahepatic obstruction to the portal circulation leads to the opening up of collateral channels A group forms at the site of the obliterated embryological veins within the falciform ligament (17) These paraumbilical veins connect the left branch of the portal vein with the superficial veins around the umbilicus In the presence of portal obstruction reasonable mixture of the blood in the right and left branches of the portal vein probably occurs It is suggested that, in the present patient, some of the blood from the left branch of the portal vein entered a paraumbilical vein and passed in the falciform ligament to emerge through a small paraumbilical hernia and join the superficial epigastric vein This vein inferiorly joins the great saphenous vein and superiorly communicates with the lateral thoracic veins, tributaries of the axillary vein (3) It is this latter communication which is seen in the venogram (D) Total blood flow through the liver in man is estimated at 1,085 to 1,845 c c per minute (2), of this one-quarter to one-eighth is from the hepatic artery The collateral abdominal vein was estimated to carry 360 c c per minute It can be assumed, therefore, that the anastomotic channel was carrying a half to a quarter of the portal venous blood directly into the right great saphenous vein This short-circuiting of portal blood and the mixing of the two streams in uncertain proportions prevented studies of hepatic function being included Observations were confined to study of the contents of the portal collateral vein during absorption from the intestine Although in 1877 the portal vein was first punctured in animals for this purpose (19) biochemical analysis of portal vein blood does not hitherto seem to have been attempted in man

In the rat and cat, glucose absorption proceeds more rapidly than that of lævulose (6, 13), similar conclusions have been indirectly reached in man (16) The present observations support this suggestion, the highest concentration of glucose in the abdominal vein preceding that for lævulose, indicating a different rate for the absorption of glucose and lævulose The interval of 30 minutes between samples prevents more accurate localisation of the actual time of maximum sugar concentration The mixing of the abdominal collateral with the systemic venous system makes it impossible to calculate the quantities of each sugar absorbed in unit time The present technique therefore suffers from the same disadvantages as the systemic blood sugar tolerance test, as concentrations rather than absolute quantities are under consideration It is believed that glucose is absorbed by a phosphorylation mechanism (23) but that the hexose phosphoric acid in the intestinal mucosa is immediately changed into hexose again and that the sugar passes as such into the blood stream The failure of the ester phosphate concentration in the portal collateral vein to increase during the absorption

of glucose is in keeping with the hypothesis The absorption of the sugar, moreover, occurred without any significant change in the serum potassium concentrations of either vein

The partition theory of fat absorption (9, 10, 12) has not been universally accepted (4, 14, 24, 25) It was hoped that observations on the present case might have shown definitely whether fat, split or unsplit, was absorbed into the portal venous system However, on two occasions fat tolerance tests using triglyceride fat showed neither systemic nor an anterior abdominal vein lipæmia The fat was introduced directly into the duodenum, the passage of the meal was not hurried and the patient did not have increased fæcal fat concentrations No explanation can be offered for the failure to demonstrate a venous lipæmia It has been shown that if neutral fat is given with lipase a portal rather than a systemic lipæmia results (11) In our patient when neutral fat was given with lipase and was presumably hydrolyzed, only then was there some absorption into the portal collateral vein However, the increase was not sufficiently conspicuous to be entirely conclusive It might have been more striking if the observations had been continued for three rather than two hours after the fatty meal

An increase in the nitrogen content of the portal vein during protein digestion has been shown in animals (7, 15, 22) In the present case, although it would have been more satisfactory to have estimated amino acid nitrogen rather than non-protein nitrogen, the increased non-protein nitrogen in the abdominal collateral after a protein meal seemed to confirm these findings, especially as the urea nitrogen concentration remained relatively constant

Many other studies will spring to mind which would have been attempted if untimely complete thrombosis of the abdominal vein had not occurred After this mishap fresh veins developed over the right flank, but these new collaterals were multiple, small and not susceptible to puncture It would also have been more satisfactory if the complication of portal cirrhosis had not been present and if more frequent venous sampling had been possible The present observations are reported in the hope that others may perhaps be more fortunate when a similar clinical opportunity presents A portal communication can readily be distinguished from a systemic anastomosis by simultaneous puncture of the vein and an arm vein one hour after a glucose drink The samples are analysed for glucose Three other patients with dilated abdominal veins have been studied by this means In each instance the sugar content of the arm and abdominal vein was identical In these cases, therefore, the abdominal vein communicated only with the greater venous circulation

SUMMARY

1 A patient with hepatic cirrhosis was studied, in whom an enlarged abdominal wall vein communicated with the portal venous system

2   After oral galactose a higher concentration of galactose was observed in a sample of blood from the anterior abdominal wall vein than in a simultaneous sample from an antecubital vein   Similar results were obtained for lævulose and glucose   The highest concentration of glucose in the abdominal wall vein appeared before that for lævulose   The absorption of these sugars into the abdominal vein was not associated with significant changes in the concentrations of serum ester phosphate or serum potassium

3   On two occasions after a neutral fat meal had been introduced into the duodenum no increased fat could be shown in the abdominal or in the antecubital vein   When neutral fat was given with lipase there was a suggestive increase in the fat content of the portal collateral vein, but the results were not conclusive

4   After a protein meal a conspicuous difference existed between the non-protein nitrogen contents of the abdominal vein and that of the systemic vein   Plasma urea nitrogen remained constant

5   A simple biochemical method of confirming the clinical diagnosis of a portal anastomotic vein is suggested

APPENDIX

The following methods were used —

Serum urea, serum non protein nitrogen   King, Haslewood and Delory, Lancet, i, 886

Serum proteins, serum potassium   King, Haslewood, Delory and Beall, Lancet, 1942, i, 207

Serum total " acid soluble " phosphate and serum inorganic phosphate   King, Biochem J, 1932, 26, 292

The ester phosphate is obtained by subtraction of the inorganic phosphate from the total acid soluble phosphate

Serum bilirubin   Haslewood and King, Biochem J, 1937, 31, 920

Serum alkaline phosphatase   King and Armstrong, Canad Med Assoc J, 1934, 31, 376

Serum cholesterol   Sackett, J Biol Chem, 1925, 64, 203

Blood sugar   Haslewood and Strookman, Biochem J, 1939, 33, 920

Blood lævulose   Herbert, Biochem J, 1938, 32, 815

Total serum fatty acid and opalescence of serum   Dixon, Drew and Samuel, 1946, in press

Lipoid phosphorus   Man and Gildea, J Biol Chem, 1936, 117, 183

Non phospholipid fatty acid was calculated by the method of Man and Gildea   J Biol Chem, 1932, 98, 43

Neutral fat was evaluated as glyceryl trioleate corresponding to the non phospholipoid fatty acid

Urine galactose   King and Aitken, Lancet, 1940, ii, 543

Urine hippuric acid   Weichselbaum and Probstein, J Lab and Clin Med, 1931, 24, 636

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Yours truly
Thomas Cruz
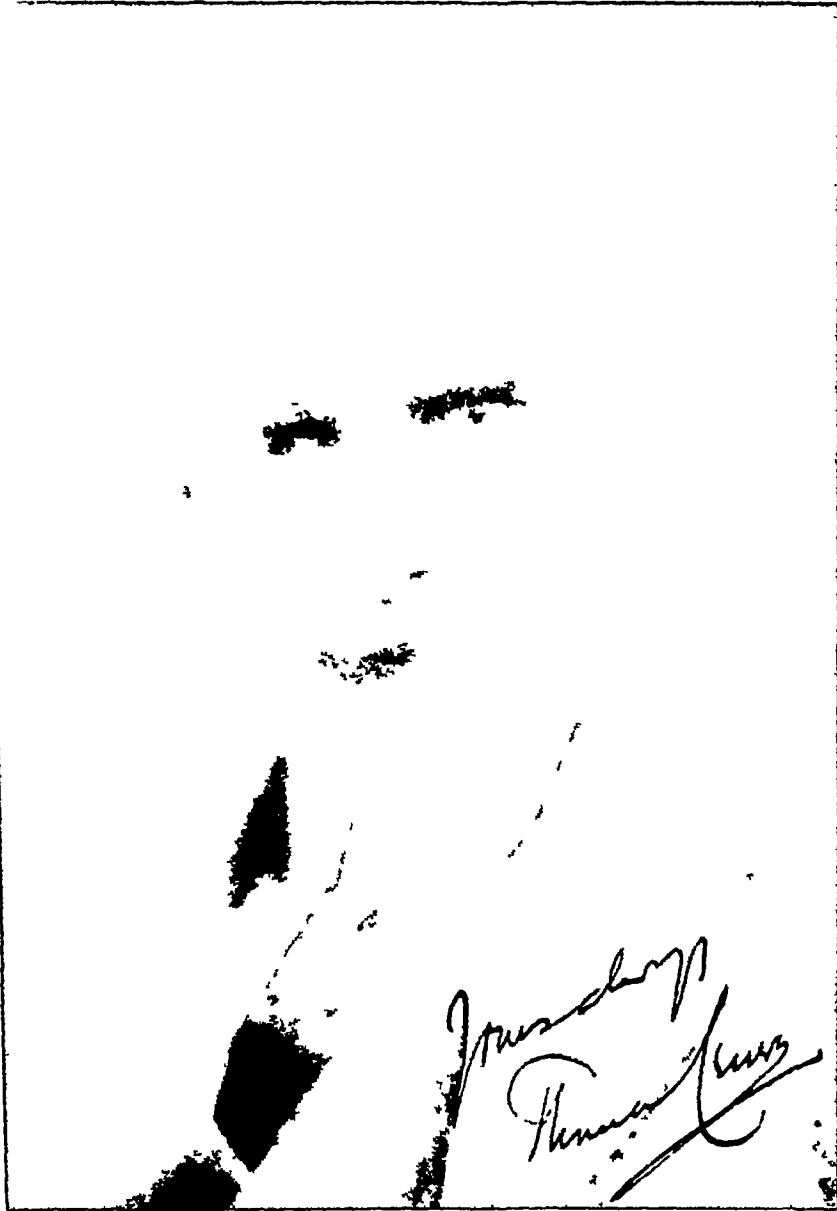
THE CIRCULATORY ACTION OF THEOPHYLLINE ETHYLENE DIAMINE

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THERE is still considerable controversy on the nature of the action of
theophylline and other xanthine derivatives on the heart   Claims are
made that these substances act as coronary vasodilators (4, 6) and as
cardiac stimulants (13, 14)   The commonly used theophylline ethylene
diamine preparation has a well-defined effect in the abolition of Cheyne-
Stokes breathing which has been the subject of much previous study   It
has been shown that this respiratory action of the drug is the result of a
stimulating action of the ethylene diamine component on the respiratory
centre, but the part played by theophylline remains uncertain (11)   In
many cases of heart failure, theophylline ethylene diamine has a pronounced
effect on right auricular pressure and cardiac output   The results obtained
in normal subjects as well as in various forms of heart failure are presented
in this paper

Methods

The methods used were those described in previous papers (9, 10),
right auricular pressure and cardiac output being measured by cardiac
catheterisation   Arterial and venous samples were analysed for their
oxygen unsaturation in a Haldane blood gas apparatus, while the oxygen
consumption was determined by spirometry, after which cardiac output
was found by the application of the Fick principle

Theophylline ethylene diamine was given intravenously in doses of
0 48 g   Equivalent quantities of theophylline were given as intravenous
doses of 0 6 g theophylline sodium acetate dissolved in 10 c c water
Ethylene diamine, when given separately, was administered in a dose of
0 1 g in 10 c c water

TABLE I

Theophylline on normal circulation

Case	Sex and age	RAP		CO l/min		Heart rate		BP		Time, mins*	REMARKS
		Before	After	Before	After	Before	After	Before	After		
VII 43	M 24	-30	-45	7 6	8 15	70	72	104/64	108/80	9	See Fig 1
VIII 140	M 27	-35	-55	10 2	15 6	60	70	135/85	140/75	10	CO back to original level in 15 min RAP still -55
VIII 133	M 20	-40	-50	8 4	10 9	68	80	125/80	126/70	2	CO back to base line in 15 mins and VP -45

* Time after injection at which the tabulated effects (maximal) was observed

ACTION OF ETHYLENE DIAMINE

Ethylene diamine was given alone to one normal subject, two patients with hypertensive heart failure, one with aortic stenosis, and a typical result is illustrated in Fig 5 from a patient with mitral stenosis and aortic incompetence The drug may produce a transient fall in venous pressure which passes off within ten minutes The other cases showed similar results There were no significant changes in heart rate or arterial pressure Such slight changes in cardiac output as were seen could be readily explained as a secondary consequence of the venous pressure fall

The main action on the circulation of theophylline ethylene diamine is therefore due to theophylline The effects of the latter drug alone and that of theophylline ethylene diamine linkage can thus be considered together
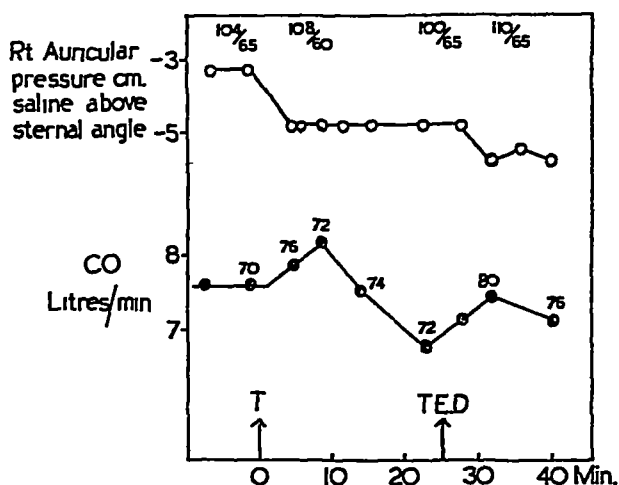
Fig 1   In this and subsequent figures B P readings are noted along the top while heart rates are recorded beside the cardiac output figures

Normal subject   Theophylline produces a fall in right auricular pressure accompanied by a transient rise in cardiac output The latter settles down to a level below the original value while the reduction of filling pressure persists Similar results follow a further injection of theophylline ethylene diamine

ACTION OF THEOPHYLLINE AND THEOPHYLLINE ETHYLENE DIAMINE

1   *Normal subjects*

Results are shown in Table I, with an example in Fig 1 It is seen that the venous filling pressure is lowered and the cardiac output shows an immediate rise which passes off within 10-15 minutes Thereafter, if the venous pressure remains low, the cardiac output may reach a level lower than that at which it began

2   *Hypertensive heart failure*

The most striking effects were seen in cases of hypertensive heart failure
Typical examples are seen in Fig 2 and the later parts of Figs 3, 4 and 7,
while the cardiac effects in the whole series are summarised in Table II
There is a rapid fall in the raised right auricular pressure with a conspicuous
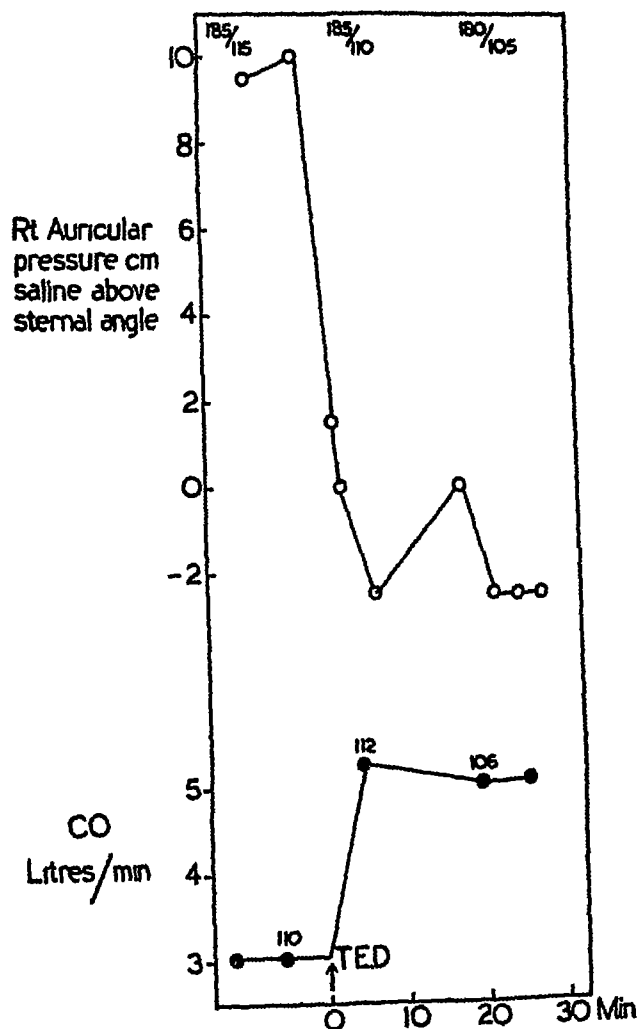
Fig 2   *Hypertensive failure*   Theophylline ethylene diamine reduces the high venous pressure
to nearly normal, while cardiac output rises from a low to a normal level

increase in the output of the heart   As a rule these effects come on inside
five minutes and are thus much more rapid in onset than the similar changes
seen after administration of digoxin, which take about 20 minutes to become
, manifest (10)
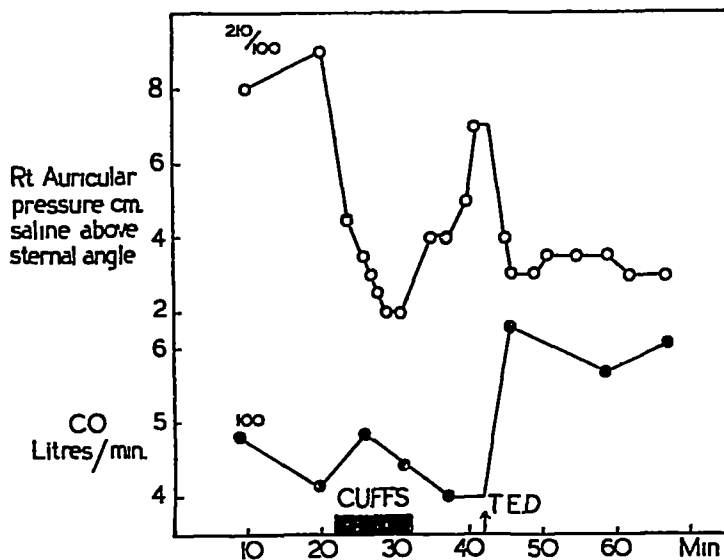
Fig 3 *Hypertensive failure* Comparable reductions of venous pressure are produced by congesting cuffs on the thighs and theophylline ethylene diamine The rise in cardiac output resulting from the latter is much greater
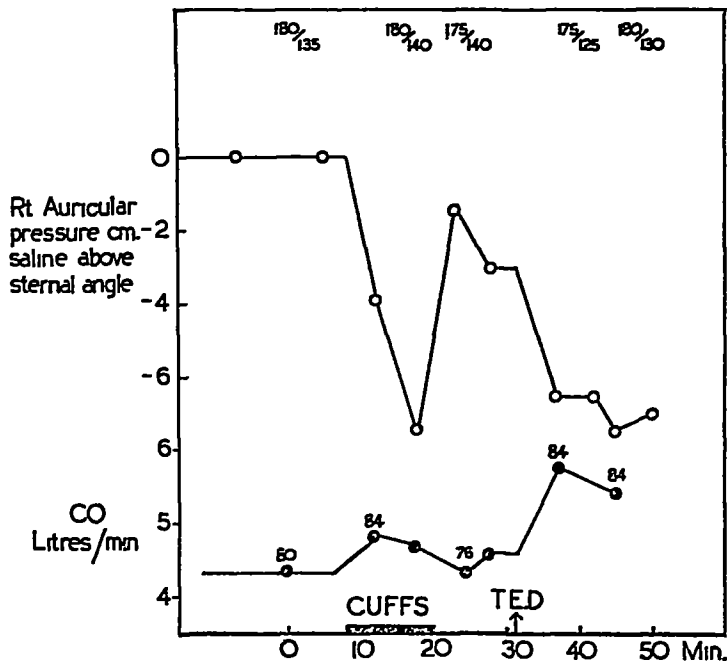
Fig 4 *Hypertensive failure* Comparable reductions of venous pressure are produced by congesting cuffs on the thighs and theophylline ethylene diamine The rise in cardiac output resulting from the latter is much greater

In previous papers (8,10) it has been shown that in cases of hypertensive heart failure, lowering of the venous pressure by congesting cuffs on the thighs, or by venesection, leads to an increased cardiac output of much the same order as that which follows similar degrees of lowering of the venous pressure produced by intravenous digoxin   When the same type of comparison was made with theophylline, cuffs on the thighs produced a small but significant increase in cardiac output, while theophylline ethylene diamine produced a greater effect, the cardiac output rising to a much higher level (Figs 3 and 4)
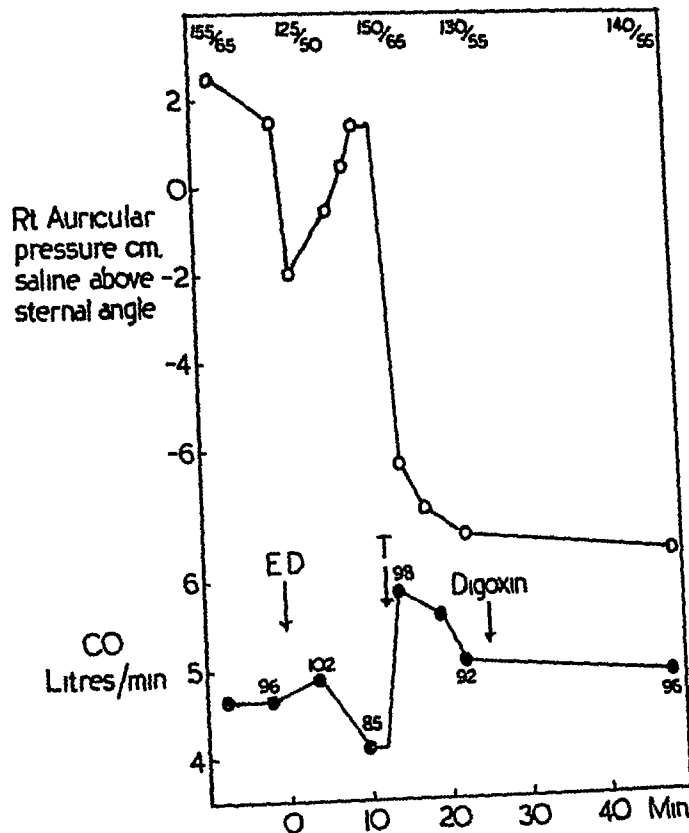
Fig 5   *Mitral stenosis and aortic incompetence*   Ethylene diamine produces only a transient effect on venous pressure   Thereafter theophylline produces a considerable rise in cardiac output which, however, quickly falls to a lower level although the accompanying fall in right auricular pressure is maintained   The transient stimulating action on the heart and the separate venous pressure reducing action are well seen

3   Rheumatic heart disease

In mitral stenosis the effects of theophylline ethylene diamine are less striking than in hypertensive failure (Table II)   While the venous pressure may fall considerably, the rise in cardiac output is less obvious (Fig 6)

4 *Paroxysmal orthopnœa (cardiac asthma)*

In two hypertensive subjects and one case of aortic incompetence there have been attacks of severe orthopnœa during the observations   In two instances the venous pressure, which had risen to a very high level in the attack, dropped rapidly to a low level after theophylline with recovery of the cardiac output and symptomatic relief   In the third (hypertension) there was no immediate response to the first injection, but a slight response of the kind just outlined (some improvement) after a second dose
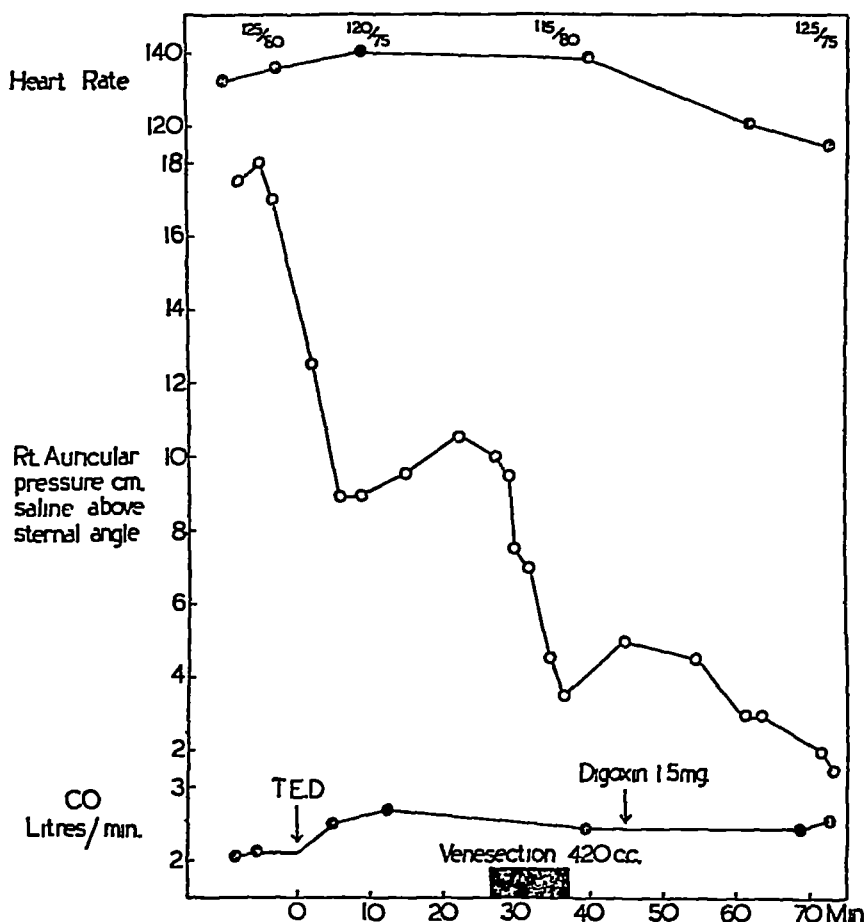
Fig 6   *Mitral stenosis with auricular fibrillation and severe failure*   Theophylline ethylene diamine produces only a slight increase in cardiac output   Further reductions in venous pressure by venesection and digoxin are not accompanied by any further increase in output

5 Emphysema

Two cases of emphysema were given theophylline ethylene diamine In one the cardiac output of 7 litres/min was increased to 7 9 litres/min , but subsequently fell to 4 litres, when the right auricular pressure, initially 7 cms below the sternal angle, fell to 11 cm saline below the sternal angle The second case had a normal right auricular pressure which fell 3 cm and the cardiac output fell from 5 7 to 5 1 litres/min
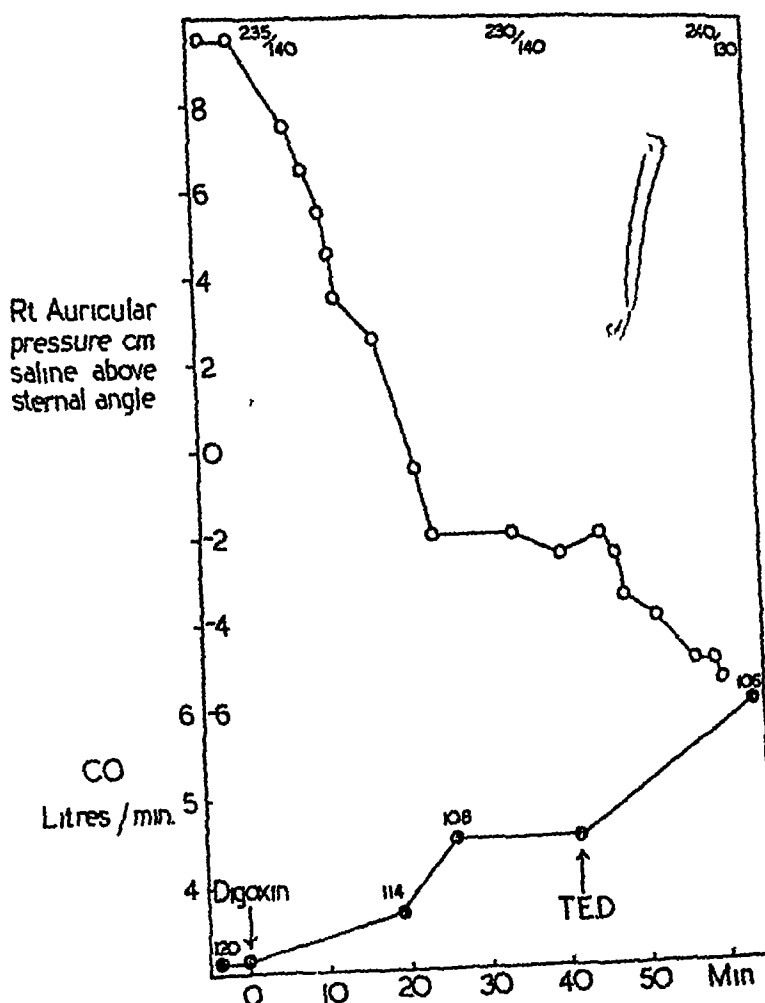
Fig 7   *Hypertensive failure*   1 5 mg digoxin i v produces an increase in cardiac output and reduction of filling pressure   Further changes in the same direction are produced by theophylline ethylene diamine

Summation of action of digitalis and theophylline

In some cases of hypertensive heart failure there may be produced a therapeutically useful summation of effects on the heart of digitalis and

theophylline ethylene diamine (Fig 7)   This effect was not demonstrable in mitral stenosis (Figs 5 and 6)

TABLE II

Effects of theophylline ethylene diamine on cardiac output (in litres per min)

A. Hypertensive heart disease										Average
Initial C O	3 0,	2 3,	2 1,	4 0,	5 1,	4 6,	4 2,	5 0,	2 2	3 6
Maximum C O after T E.D	5 5,	3 6,	2 8,	6 3,	6 8,	5 4,	4 9,	7 3,	3 5	6 1
Increase	2 5,	1 3,	0 7,	2 3,	1 7,	0 8,	0 7,	2 3,	1 3	1 5

B Mitral stenosis					Average	
Initial C O	2 75,	2 15,	4 1,	5 4,	3 05	3 5
Maximum C O after T E.D	2 5,	2 7,	5 9,	5 1,	3 5	3 95
Change	—·25,	+ 55,	+1 8,	—·3,	+·45	+ 45

DISCUSSION

The results show that the main action of theophylline ethylene diamine on the circulation is due to its theophylline component   Greene, Paul and Feller (7) noted that theophylline ethylene diamine reduced the venous pressure in normal subjects and in patients with congestive heart failure Analysis of our results agrees with their findings but suggests that there are other actions of theophylline in addition to those of venous pressure reduction and perhaps more complex than those of digitalis

Evidence of a stimulating action on the heart   In normal subjects both digitalis and venesection lower right auricular pressure which results in a decrease in cardiac output (9, 10)   Theophylline may be followed by a short phase of increased output in normals, in spite of a lowered right auricular pressure   In severe low output heart failure, the heart responds to a fall in filling pressure by an increase in output   The increase in output after theophylline is, however, greater than that produced mechanically by cuffs on the thighs (Figs 3 and 4)   This suggests that theophylline ethylene diamine has some immediate direct stimulating action on the heart which is certainly much more easily demonstrable than any comparable

action of digitalis   The possibility that increases in cardiac output after theophylline are due to acceleration of the heart have been considered, but the increases in rate which occur seem too slight and irregular to play any significant part   The stimulating action of theophylline in normal, emphysematous, and rheumatic subjects has been noted to be less certain and more fleeting than that usually seen in hypertension   This may explain some previous failures to detect this cardiac action in man and animals by older methods (11, 13)

Starr and Gamble (14), using the ballistocardiograph, found a stimulating action of theophylline on the heart   Stewart and Jack (15) also observed that the peripheral blood flow was increased by the drug, and supported Starr and Gamble's interpretation   Boyer and his collaborators (1, 2, 3), in a study of the action of theophylline on the coronary blood flow, came to the conclusion that the increase in flow which took place was in large part accounted for by an adrenaline-like action on the heart, increased myocardial activity leading to an increased coronary flow as a consequence of the metabolic demands of the myocardium   The stimulating action on the heart seen in our observations is certainly somewhat similar to that which has been demonstrated in the normal human heart with minute doses of adrenaline (9), and we would agree with Boyer in attributing an " adrenaline-like " action to theophylline so far as the heart is concerned

Numerous attempts have been made to demonstrate a coronary vaso-dilator action of theophylline   Evans and Hoyle (5) could demonstrate no clinical benefit in cases of angina pectoris   Efforts to show that the drug modified the myocardial degeneration following ligation of coronary arteries have not been strikingly successful (6)   Merrill (12) records the induction of cardiac pain and sudden death after the injection of aminophylline in coronary thrombosis, and the stimulating action of the drug may well add to such risks   Although the type of observation we have reported here cannot settle the question of a coronary dilator action, it certainly seems unlikely that coronary vasodilatation plays any causal part in the production of the more vigorous cardiac contractions which follow the administration of theophylline

Venous pressure reduction has been demonstrated to take place when the cardiac output increase is slight (Fig 6), and to persist when the cardiac stimulation has passed off   It is therefore a separate action and not accounted for by the cardiac augmentation   Possible mechanisms of the venous pressure fall have been discussed previously (10) and we can only postulate a reduction in venomotor tone as a possible mechanism   The fall in right auricular pressure produced by theophylline is usually very rapid, and it is this rapidity of action which is one of the greatest advantages of the drug, since the maximum effect after intravenous digoxin may only be produced after twenty minutes, and very rapid venesection may be difficult in severe heart failure   As this effect of theophylline on right

auricular pressure may not be maintained, a combination of the drug with digitalis, after which the venous pressure fall tends to persist, has been demonstrated to give a summation of effects In practice, therefore, theophylline ethylene diamine may be of the greatest value in cases in which a decrease in auricular pressure is urgently required

Summary

1   The circulatory action of theophylline ethylene diamine is due to its theophylline component, ethylene diamine having only a very slight and transient venous pressure reducing action

2   Theophylline reduces the venous filling pressure of the heart and also has a more transient direct stimulating action on the heart increasing its output

3   In normal subjects there may be a transient rise in cardiac output followed by a fall below the initial level as the stimulating action passes off and the lowering of filling pressure persists A similar reaction is also seen in emphysema

4   In hypertensive failure the combination of venous pressure lowering action and cardiac stimulation leads to remarkable improvements in cardiac output which in the first 15-30 minutes after injection of the drug are greater than those seen after digoxin injections Digoxin and theophylline may sometimes be given in close succession with summation of their actions

5   In mitral stenosis with heart failure the venous pressure falls but the accompanying rise in cardiac output is less than in hypertensive heart failure

ADDENDUM

Since this paper was submitted for publication Smith and Jensen (J Lab Clin Med 1946, 31, 850) have reported that theophylline amino isobutanol stimulates myocardial contraction in the normal or failing heart lung preparation Using the same compound on clinical cases Steinberg and Jensen (*ibid* 1946, 31, 857) also noted a rapid fall in venous pressure and shortening of the circulation time, the action lasting for 60 90 minutes

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THE SURVIVAL OF TRANSFUSED ERYTHROCYTES, WITH SPECIAL REFERENCE TO CASES OF ACQUIRED HÆMOLYTIC ANÆMIA *

By P L MOLLISON

A DIRECT method for investigating the fate of erythrocytes after transfusion from one animal to another was first introduced by Todd and White (24) The method depended upon the use of specially prepared immune hæmolytic sera which reacted specifically with the erythrocytes of a given Bull (A) but not with those of other Bulls, so that after transfusion of blood from Bull A to another (B), the presence of A erythrocytes in the blood stream of Bull B could be demonstrated by testing the mixed blood with the " anti-A " serum

Ashby (1) first applied this principle to the determination of the survival of transfused erythrocytes in man Use was made of the practice of transfusing group O blood to recipients of other groups Thus in the case of a recipient of group A for instance, the mixture of O and A erythrocytes in the recipient's blood after transfusion could be analysed by agglutinating out the A erythrocytes with an anti-A serum and then counting the group O erythrocytes

During the twenty years that followed the publication of Ashby's first paper only sporadic interest was taken in the method In 1940, however, Wiener and Schaefer (30) demonstrated its value in assessing the time for which erythrocytes could usefully be stored, and thus reawakened interest in the subject Since then, there has been an increasing realisation, firstly that reliable quantitative results can be obtained with the Ashby technique or some modification of it and secondly that the method enables one very simply to obtain most useful evidence in a variety of hæmatological problems

For instance, it has been demonstrated that estimation of the survival in vivo of the erythrocytes of stored blood is the only reliable criterion of their viability, since the commonly accepted in vitro tests are often false guides (15, 22, 23), the solution of problems of compatibility, particularly those involving the Rh types, has been greatly facilitated by contrasting the survival of blood of different types in different recipients (31, 29, 18), the question of the normal life span of the erythrocyte, deduced from the survival of transfused erythrocytes, has been considered by Wiener (28) and Callender, Powell and Witts (5), and others, evidence regarding red cell destruction in various types of hæmolytic anæmia has been obtained by Dacie and Mollison (7), Mollison (19) and Brown, Hayward, Powell and Witts (4), the method has also been used to measure blood volume as a check on another method of estimation (17)

The chief purpose of the present paper is to describe the results of survival experiments in some cases of acquired hæmolytic anæmia At the same time the opportunity will be taken of presenting some hitherto unpublished results of survival experiments in patients without hæmolytic anæmias and these will serve as a contrast to illustrate the features of normal survival

Methods

The procedures followed were those described previously (23), concentrated erythrocyte suspensions, prepared from blood stored for not more than four days in a citrate-glucose solution, being used for transfusion Except in a few special experiments, only blood from healthy donors was used

The survival of donor cells was estimated by a modification (7) of Ashby's Method (1) Patients of group A or B were transfused with group O blood and a group O serum was used for differential agglutination In a few cases, patients of group O were transfused and differential agglutination was then effected with an anti-Rh, anti-M or anti-N serum, donors of appropriate Rh and MN type being selected

The technique of differential agglutination has been very slightly further modified In particular, very powerful group O sera exhibiting "zoning" are now used and preliminary tests are set up to discover the dilution in which agglutination of the recipient's erythrocytes is most nearly complete This dilution is then used throughout the experiment

The complete technique is then as follows 20 c mm of blood from a well mixed, oxalated venous sample is pipetted into 2 c c of serum-saline mixture (of optimal dilution) in a small test-tube (5 × 1 cm) and after mixing left at room temperature for not less than one hour The tube is then centrifuged at about 1,000 r p m for just sufficient time (about one minute) to cause loose packing of the cells with a clear supernatant fluid The tube is then shaken with moderate vigour and immediately re-centrifuged

After three centrifugings and three mixings the tube is held upright for a few seconds to allow the large clumps to start sedimenting and then immediately a drop of fluid is removed with a Pasteur pipette from the top of the column (now free from large agglutinates) and placed on a counting chamber Usually two "spreads" are made and a total of 1,000 cells counted For modifications when using anti-M or anti-N sera see (7), when using anti-Rh sera, the centrifuge technique was employed but only 0 2 c c quantities of serum and blood suspension were used, for the sake of economy

Expression of results

The first workers (1, 26) to publish the results of experiments made with the differential agglutination method reported their results directly, in terms of the absolute concentration of un-agglutinated erythrocytes surviving in the recipient's circulation at different intervals after transfusion Considerable emphasis was laid on the " total survival time," *i e*, the maximum time during which donor erythrocytes could be detected in the recipient's circulation, and this time was used to summarise the results of each experiment Martinet (16) and Dekkers (9) also tried to estimate the total survival time However, direct determination of the point at which donor erythrocytes finally disappear from the recipient's circulation presents technical difficulties, since the method is least sensitive when there are fewest surviving erythrocytes

Before considering how this problem could be overcome (for example, by transfusing much larger quantities of blood than those used by the workers mentioned), it is worth enquiring whether the total survival time is in fact an adequate expression of the results The answer to this question depends upon whether or not the number of erythrocytes surviving at intervals after transfusion is found to be inversely proportional to the time since transfusion , that is to say, when the results are plotted graphically, whether the slope of elimination is linear If the slope were linear under all conditions, not only would calculation of the total survival time be the most satisfactory method of comparison between one case and the next, but direct estimation of the time at which donor erythrocytes were last present in the circulation would be unnecessary since this point could be readily determined by extrapolation of the slope formed by plotting intermediate estimates

In practice, as first pointed out by Witts and his co-workers (4), the slope of elimination is not always linear In hæmolytic anæmias the slope may be curved and, although a linear slope is the rule in subjects not affected with a hæmolytic process, an initial curvature followed by strict linearity may be found in subjects rendered plethoric by transfusion (4) Again, after transfusions of old stored blood there may be an initial phase of rapid destruction followed by a phase of slower destruction (21, 23) In such cases the total survival time does not adequately describe the results and some information about the proportion of erythrocytes surviving at various

intervals after transfusion must be given The simplest method of doing this is to plot the results on a graph, which can be submitted to mathematical analysis (4) Alternatively, the percentage survival at different arbitrarily chosen intervals can be calculated and used as a basis of comparison between one case and the next

One difficulty deserves discussion at this stage and that is consideration of blood volume changes after transfusion Following the transfusion of blood to a recipient with a previously stable blood volume the latter is temporarily increased and is only slowly re-stabilised Because of this disturbance there is usually an initial progressive increase in the count of donor erythrocytes in the recipient's circulation and the maximum figure may not be reached until 48 hours after transfusion Since there is but little information available about the duration and extent of these changes, the following figures, although incomplete, are given here

Two groups of recipients were tested Four subjects received an average of 500 c c of concentrated erythrocyte suspension in 20 to 40 minutes, and eight other subjects received an average of 1,010 c c of citrated blood in periods varying from 20 to 85 minutes

In both groups the venous samples obtained five minutes after the end of transfusion were tested The concentration of donor erythrocytes and the hæmoglobin and hæmatocrit values of this sample were each taken as 100% and the figures for estimations on samples taken at 24 and 48 hours after transfusion were expressed as percentages of these figures The results are shown in the following table —

TABLE I

Changes in concentration of donor cells and in hæmoglobin and hæmatocrit values during 48 hours after transfusion

(Taking as 100% the values found immediately after transfusion)

Case No	Transfusion fluid*		Percentage at 24 hours			Percentage at 48 hours		
	Age	Amount	Donor Cells	Hb	Hmt	Donor Cells	Hb	Hmt
1	1	510 (C)	116	103	112	115	103	112
2	3	510 (C)	99	108	105	100	104	107
3	3	515 (C)	111	117	117	111	126	127
4	3	490 (C)	109	106	—	114	112	—
		Averages	108 7	108 5	111 3	110 0	111 2	115 3
5	0	1040 (B)	102	106	108	—	—	—
6	0	1000 (B)	109	112	130	117	115	118
7	0	950 (B)	—	—	—	109	112	109
8	0	1050 (B)				108	120	116
9	2	1050 (B)	116	109	106	101	112	106
10	2	995 (B)	102	112	112	108	104	107
11	2	1035 (B)	120	108	108	106	111	116
12	4	970 (B)	107	110	110			
		Averages	109 3	109 5	112 3	108 3	112 3	112 0

* The citrated blood (B) had an average red cell count of 3,760,000 per c mm , and the concentrated erythrocyte suspensions (C) an average of 7,000,000 per c mm

It will be observed that in both groups there is a substantial increase in all the values during the 24 hours following transfusion, indicating a compensatory decrease in blood volume during this period   The changes in the period 24-48 hours after transfusion are much smaller in extent, suggesting that as a rule changes are mainly completed within the first 24 hours

In Table II some figures are given from three cases in which further estimates were made of the concentration of donor erythrocytes in the recipient's circulation at 72 hours after transfusion   It appeared that the blood volume in these cases had become stabilised within 48 hours of transfusion

<div align="center">TABLE II</div>

Changes in hæmoglobin, hæmatocrit and donor cell concentration during the 72 hours after transfusion in three recipients

	Hb	Hæmatocrit	Concentration of donor erythrocytes per c.mm
Case 2 Transfusion of 510 c c concentrated cell suspension			
Before transfusion	48	27 4	—
After transfusion	60	32 8	1,086,500
24 hrs after transfusion	65	34 4	1,076,000
48 hrs after transfusion	62	35 0	1,092,000
72 hrs after transfusion	63	35 2	1,075,000
Case 3 Transfusion of 515 c c concentrated cell suspension.			
Before transfusion	29	17 0	—
After transfusion	36	21 4	843,000
24 hrs after transfusion	42	25 0	938,000
48 hrs after transfusion	45	27 0	939,000
72 hrs after transfusion	44	25 5	866,000
Case 13 Transfusion of 845 c c concentrated cell suspension			
Before transfusion	39	17 9	—
After transfusion	72	31 0	1,599,000
24 hrs after transfusion	74	31 4	1,721,000
48 hrs after transfusion	74	34 0	2,021,000
72 hrs after transfusion	75	34 3	1,961,000

The evidence suggests then that blood volume changes are usually complete by the end of 48 hours after transfusion   From this evidence one can draw the conclusion that in plotting the results of survival experiments it will be best to ignore estimates falling in the first 48 hours although, particularly when only 500 c c of blood or erythrocyte suspension have been transfused, the first sample may be taken at 24 hours without introducing any large error   Of course, such an estimate taken at an appreciable interval after transfusion will not represent 100% survival because there will already have been some destruction of donor erythrocytes

Experiments in which the prospective recipient is submitted to venesection before transfusion, so that a sample taken shortly after transfusion

represents 100% survival with minimum error, support the idea that the
rate of destruction is normally no more rapid in the immediate post-
transfusion period than it is subsequently (Case 25 and Callender and
others (5))

When the rate of destruction is very rapid, as in the case of some
hæmolytic anæmias, a sample taken immediately after transfusion may give
the best estimate of the maximum donor cell count

In the cases reported in the present paper, samples were usually taken
immediately after transfusion and then at 24 or 48 hours and subsequently
at suitable intervals depending upon the expected rate of destruction   When
the rate of elimination was normal or approximately normal the count of
donor erythrocytes in the 24 or 48 hour sample was taken as the first estimate
for plotting graphically, or was considered as 100% survival when cases
were being compared on this basis   When destruction was rapid, the count
of donor erythrocytes in the sample taken immediately after transfusion
was taken as 100% although it is realised that for accurate analysis of the
curves, the estimates falling in the first 24 hours at least are subject to
slight correction

In representing the results graphically, straight or curved lines have
been drawn to pass as closely as possible to the majority of the points

RESULTS

SECTION I   CASES SHOWING NORMAL SURVIVAL

(a) A mixed group of recipients

This group of recipients is made up of eleven patients affected with
hypochromic anæmia (idiopathic or secondary to hæmorrhage), three
convalescent subjects and one healthy subject   This group includes some
of the cases forming the control group in a previous paper (23) but some
cases followed for longer periods have been substituted and the results are
now given in more detail (Fig 1)

In calculating the figures for percentage survival the estimate at
24 or 48 hours (72 hours in one instance) has been taken as 100% survival
except for Case 25 who was venesected immediately before transfusion
and in whom the concentration immediately after transfusion was taken
as 100%

It will be noted, firstly, that the slopes are approximately linear and
secondly, that the average total survival time appears to be between 100
and 120 days

This survival pattern compares very closely with that first reported
by Wiener (28) as the normal finding after transfusion   From the fact
that, in the present series, the hæmoglobin level of the recipient has no
obvious effect on the survival rate and from the recent work of Callender

et al (4) on the survival rate in healthy non-anæmic subjects, one may
conclude that any differences between the survival rate in anæmic and
non-anæmic subjects are certainly small

Whilst 14 of the 15 cases form a fairly compact group on the graph,
one case (Case 4) shows a distinctly lower survival rate and the slope of
elimination appears to be curved   The recipient was a recently delivered
woman and it was at first suspected that the shorter survival might be due
to previous sensitisation to the Rh agglutinogen   However, the patient
proved to be Rh positive and among serological factors only the much smaller
possibility of sensitisation to Rh subgroups remained   During an
investigation of the survival of stored erythrocytes (14) a few similar cases
were encountered, that is, subjects in whom the finding of a diminished
survival rate was quite unexpected and could not be explained upon
serological grounds   Further investigation has revealed that the pheno-
menon is reproducible as between a given donor and recipient but the cause
of it is still obscure (Loutit and Mollison, unpublished observations)

In Table III a few details about each of the 15 cases are recorded
together with the figures for percentage survival at 30, 60 and 90 days
which have simply been read off the graph   It is evident that neither the
hæmoglobin level of the recipient nor the short period of storage of the
donor erythrocytes has any gross effect upon survival

TABLE III

Survival of transfused erythrocytes in convalescent subjects or subjects affected with a hypochromic anæmia

Case No	Sex	Diagnosis	Hb%	Amount* trans fused (c c)	Length of storage (days)	Percentage survival at		
						30 days	60 days	90 days
3	F	Hypochromic anæmia	29	500 (C)	3	80	47	21
4	F	Hypochromic anæmia	54	490 (C)	3	48	19	—
5	F	Hypochromic anæmia	90	1040 (B)	0	59	35	7
14	F	Hypochromic anæmia	56	1250 (B)	1	80	40	17
15	F	Hypochromic anæmia	60	500 (C)	1	86	40	—
16	F	Hypochromic anæmia	50	400 (C)	1	58	36	—
17	F	Hypochromic anæmia	40	795 (C)	2	74	46	17
18	F	Hypochromic anæmia	58	970 (C)	3	70	47	17
19	F	Hypochromic anæmia	76	1010 (C)	3	70	43	4
20	F	Hypochromic anæmia	60	1000 (B)	3	71	40	—
21	F	Hypochromic anæmia	60	450 (C)	3	66	50	—
22	M	Infective hepatitis	102	990 (B)	1	72	—	—
23	M	Chronic bronchitis	102	650 (B)	2	68	46	—
24	M	Gastric ulcer	108	900 (B)	2	79	—	—
25	M	Normal subject	92	480 (C)	4	80	51	31
					Average	71 1	42 2	16 3

* B = Blood, C = Concentrated erythrocyte suspension

(b) *A group of diseased subjects*

(1) Chronic sepsis Survival was estimated in six patients suffering from some chronic infection (e g , chronic osteomyelitis, pyelitis of pregnancy, empyema thoracis, etc) In none of these cases was there evidence of a hæmolytic process from the clinical point of view or from an ordinary blood examination In each case, normal survival of the donor erythrocytes was observed (see Table IV and Fig 2)

This finding is expected in view of previous observations (25) that the ordinary anæmia of sepsis is not hæmolytic in nature

Since this work was completed, Brown and others (4) have published evidence that in chronic cases of sepsis erythrocyte destruction, as estimated by the method of differential agglutination, is normal but that in more acute septic conditions destruction may be rapid An example of this latter type is presented below (see Section 11)

(2) Carcinomatosis Two cases were investigated , in neither of these was there any evidence of actual blood loss during the period of the experiment Normal survival of transfused erythrocytes was observed in each instance (see Table IV and Fig 2)

(c) *Two cases of Addisonian anæmia (Cases No 32 and 33)*

Ashby (2) first reported finding prolonged survival of normal erythrocytes transfused to cases of Addisonian anæmia In Fig 3 are
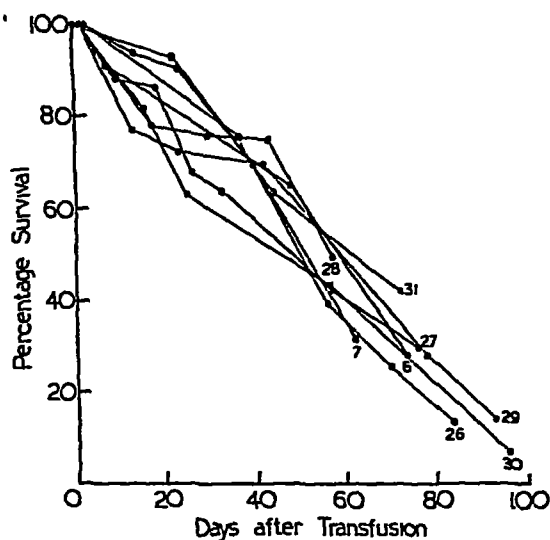
Fig 2   Survival of transfused erythrocytes in six cases of chronic infection and two cases with a carcinoma (*see* Table IV)

TABLE IV

Survival of transfused erythrocytes in patients affected with a chronic septic process or a neoplasm

Case No		Hb%	Length of storage of transfused blood	Percentage survival of transfused erythrocytes at		
				30 days	60 days	90 days
26	Chronic osteomyelitis	52	0	82	34	—
7	Chronic empyema	72	0	80	34	—
6	Chronic empyema	62	1	77	46	—
27	Chronic empyema	68	2	65	40	—
28	Pyelitis of pregnancy	66	3	71	43	—
29	Chronic pulmonary tuberculosis	60	3	71	48	16
30	Carcinoma of cervix	65	3	59	40	—
31	Carcinoma of cervix	42	4	82	50	—
				73 9	41 9	—

plotted the results of estimations made in one case   An estimate made only two hours after transfusion has been included because blood volume before transfusion was very small (approximately 1 9 litres) and there was some evidence from Hb and hæmatocrit estimations that it remained at a higher level after transfusion , for the same reason, actual figures rather than percentage survivals have been plotted   It will be seen that the slope was linear and the total survival time was a little over 90 days

A second case was only followed for six days after transfusion, when 940,000 donor erythrocytes per cu.mm   remained out of 1,060,000 per

cu mm in the 24 hour sample   This finding is consistent with normal survival

These results of course only indicate that in pernicious anæmia there is no mechanism destroying normal red cells , they do not give any information about the survival time of the red cells produced by the patient   In fact, Loutit (12) has recently demonstrated that the survival time of the latter is reduced

(d) A case of nocturnal hæmoglobinuria

Dacie and Mollison (7) reported the finding of a normal survival rate of erythrocytes from a normal donor in a case of nocturnal hæmoglobinuria and fuller details of the same case were published by Dacie and Firth (6)   The survival of transfused erythrocytes has now been estimated in a second case and normal survival has again been found   I am indebted to Professor H P Himsworth for allowing me to estimate the survival in this case and to report the results

Case 34   Mrs N S first became ill at Christmas time in 1943   She was admitted to University College Hospital in July, 1944, complaining of palpitation, pallor, dyspnœa and swelling of the ankles   Her blood count was as follows   R B C 550,000 per c mm , Hb 17%, M C D 80 μ , W B C 2,000 per c mm , reticulocytes 8% (17% shortly afterwards), osmotic fragility normal   She responded well to transfusion and was discharged from hospital   She was readmitted in November with hæmoglobinuria , blood examination revealed hæmoglobinæmia and methæmalbuminæmia , the " acid hæmolysis " test was positive and a diagnosis of nocturnal hæmoglobinuria was made

She was transfused on January the 26th, 1945, with 1,000 c c of a concentrated, washed, erythrocyte suspension prepared from two group O Rh negative and two group O Rh positive donors   The patient was group O, Rh positive and the survival of the Rh negative erythrocytes was estimated after differential agglutination with an anti-Rh serum   A slow rate of elimination was found , out of an initial total of 1,206,000 donor cells per c mm 24 hours after transfusion, 540,000 per c.mm , or 45%, remained at 55 days   This clearly indicates that in this disease, as in familial hæmolytic anæmia, there is no mechanism causing abnormal destruction of normal erythrocytes

(e) Familial hæmolytic anæmia

Dacie and Mollison (7) reported that whereas erythrocytes from an active case of familial hæmolytic anæmia had a short survival time when transfused to a normal recipient, erythrocytes from normal donors survived for a normal length of time in patients affected with familial hæmolytic anæmia   They pointed out that these findings support the hypothesis that the basic abnormality of familial hæmolytic anæmia is the formation of

erythrocytes with an increased tendency to hæmolysis and cannot be reconciled with any theory which assigns a major role to abnormal destructive mechanisms

From these observations it would be expected that normal erythrocytes when transfused to patients affected with familial hæmolytic anæmia would retain their normal fragility The following experiment was designed to test this supposition

Case 35 Sgt B, aged 29, developed an acute swelling in the L hypochondrium, followed by jaundice, and was found to be a typical case of familial hæmolytic anæmia with numerous affected relatives (Subsequent operation revealed evidence of an old hæmorrhage around an enlarged spleen) Blood examination R B C 3,600,000, Hb 63%, Hmt 24 1, M C V 77 μ^3, M C D 61 μ, reticulocytes 5%, osmotic fragility 4% lysis in 0 72% NaCl, 60% in 0 54% NaCl, Blood Group A He was transfused with 500 c c of concentrated erythrocyte suspension prepared from group O blood previously stored in citrate-glucose for four days
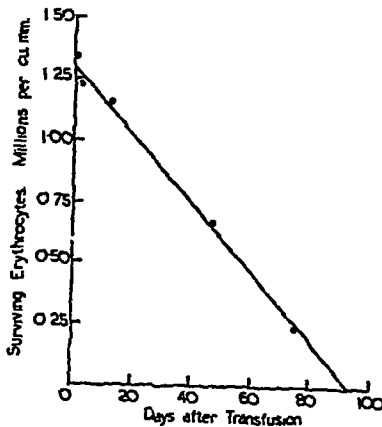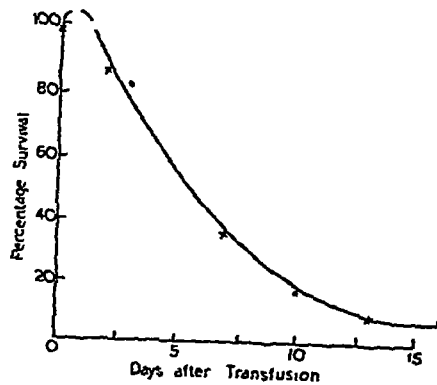
Fig 3   Fig 4

Fig 3 Survival of transfused erythrocytes in a case of Addisonian anæmia (Case 32)

Fig 4 Survival of transfused erythrocytes in a case of idiopathic acquired hæmolytic anæmia (Case 36) Estimates for second and third transfusions plotted together (X—X second transfusion   0—0 third transfusion)

On the day following transfusion blood was taken and tested by the following technique, devised by Dr M Maizels

Five c c of patient's blood were taken into 2 c c of 3% citrate, approx 6 c c citrated blood were then mixed with 7 c c potent anti-A serum and 6-7 c c saline and put into a large flask in the refrigerator for two hours, then poured into a cylinder and after ½ minute filtered through a No 4 Whatman paper The filtrate contained no agglutinates but after

centrifuging, a few small clumps were found, not increased by the addition of further potent anti-A serum , thus the great majority of the cells in the filtrate were donor cells

The filtrate was now centrifuged in special tubes so that the volume of the deposit could be measured , supernatant was then discarded and the cells resuspended in phosphate buffer to make a 1% suspension 0 05 c c of this suspension was then measured into each of a series of saline solutions and the degree of hæmolysis read in a colorimeter after 10 minutes, after centrifuging the tubes

As controls, the following bloods were also put through the entire process

(1) Patient's blood + anti-B serum (no agglutination caused filtrate contained mixture of patient's + donor's cells)

(2) and (3) Two normal bloods mixed with a compatible grouping serum

The results were as follows

Percentage lysis in following concentrations of NaCl

	0 72	0 66	0 60	0 54	0 51	0 48	0 45	0 42	0 39	0 36	0 33
Pt 's blood + anti A (i e , donor's cells extracted from recipient's circulation)	—	—	0	0	0	3	5	5	9	20	40
Pt 's blood + anti B (i e , donor's cells in recipient's circulation plus patient's own cells)	0	5	20	44	47	55	67	66	85	—	—
Pt 's blood untreated for comparison	0	5	20	50	55	60	74	78	90	—	—
Normal (Dr M) + serum	—	—	—	—	—	—	—	0	2	20	52
Normal (P L M) + serum	—	—	—	—	—	—	—	5	12	27	52

The experiment was repeated eleven days after transfusion with a similar result Incidentally, survival of the transfused cells was followed for four weeks and only a slow rate of elimination was found

Comment This experiment demonstrates that normal erythrocytes transfused to an active case of familial hæmolytic anæmia retain their normal fragility whilst circulating in the patient's blood stream This finding lends further support to the view that in familial hæmolytic anæmia the basic abnormality lies in the patient's own erythrocytes

SECTION II SURVIVAL IN CASES OF ACQUIRED HÆMOLYTIC ANÆMIA

The cases of acquired hæmolytic anæmia in this series have been arbitrarily divided into two groups, idiopathic and symptomatic In the symptomatic group have been included those patients who had a condition, for example, lymphadenoma or pulmonary tuberculosis whose occasional association with hæmolytic anæmia is well recognised In the idiopathic group have been included those cases in which hæmolytic anæmia was the central feature of the illness and in which there was no other recognisable disease Since, in fact, the reason for the occurrence of hæmolytic anæmia in certain cases of severe pulmonary tuberculosis is not in the least understood, it will be seen that the division of cases adopted here is merely one of convenience

The idiopathic cases have been divided into two groups, the first of which consists of four cases with features very like those of familial hæmolytic anæmia (acholuric jaundice), the second group consists of a single case whose features suggest that different ætiological factors were concerned

(a) Idiopathic types

(1) *Cases of acquired idiopathic hæmolytic anæmia, closely resembling familial hæmolytic anæmia (acholuric jaundice)* Observations were made upon four cases All were middle-aged or elderly subjects with a history of only a few months of definite illness, of which the most constant symptoms were weakness and dyspnœa All exhibited pallor with a slight yellow tinge and in every case the spleen was enlarged A blood examination showed a severe anæmia of macrocytic type, but with a proportion of spherocytes, reticulocytes were numerous and osmotic fragility of the erythrocytes considerably increased The serum bilirubin concentration was increased and Schumm's test for methæmalbumin (10) was positive, although no methæmalbumin could be demonstrated spectroscopically No hæmolysins could be demonstrated in the sera Only the history of previous good health, extending over forty years or more and the absence of any family history of jaundice or anæmia suggested that these were cases of acquired rather than familial hæmolytic anæmia In every case rapid elimination of the transfused erythrocytes was observed

Case 36 Female (M S), aged 60 This patient first presented herself complaining of general weakness for the previous fortnight following " a bad attack of jaundice " Her skin had a pale lemon colour and her spleen was enlarged A blood examination showed R B C 2,100,000 per c mm , Hb 50%, C I 1 2, hæmatocrit 21 , M C V 100 μ^3 , reticulocytes 32% , plasma bilirubin 2 4 mg per 100 c c , direct Van den Bergh positive ,

Schumm's test positive, osmotic fragility considerably increased (ı e , lysis commenced in 0 75% NaCl , 50% lysis in 0 46% NaCl , 100% lysis in 0 36% NaCl) A blood film showed marked anisocytosis of the red cells, with a fair number of macrocytes and some spherocytes , polychromasia and punctate basophilia W B C 4,000 per c mm (Polymorphs 65%, lymphocytes 32%, eosinophiles 1%, basophiles 1%, monocytes 1%) Normoblasts 1,500 per c mm Marrow puncture leuco-erythroblast ratio 1 1, (red cell series , haemocytoblasts 2, erythroblasts 11, normoblasts 37) Whole blood samples showed an increased rate of autohaemolysis in vitro

There was slight autoagglutination at 5°C but none at room temperature

This patient had been in hospital four years previously for a long standing varicose ulceration of the leg A blood count at that time showed R B C 4,800,000 per c mm Hb 84%, reticulocytes 0 4% This blood picture together with a history of perfect health previously and the absence of any familial history of jaundice or anaemia was considered to make it most improbable that this was a familial case of haemolytic anaemia The varicose ulceration healed after periarterial sympathectomy

Four consecutive blood transfusions were given and on each occasion there was a rapid elimination of the transfused erythrocytes (see Table V) There were no gross variations in the rate of elimination between one transfusion and another However, the erythrocytes of the first transfusion were eliminated rather more rapidly than those of the subsequent transfusions There was a close similarity between the survival figures for the second and third transfusions and the estimates, expressed as percentages of the counts immediately after transfusion, have been plotted together in Fig 4* to show the similarity and demonstrate the curvature of the slope of elimination The curve does not start smoothly from the first estimates and the initial, " hump " evidently expresses the tendency to an increase in the count of donor erythrocytes, consequent upon blood-volume readjustment, as has previously been discussed

In the case of the fourth transfusion, erythrocytes of different serological types (ARh − and O Rh +) were given immediately one after the other to see whether an α_1 agglutinin in the patient's serum, weakly active at room temperature, would have any effect on the rate of elimination of the A erythrocytes As expected, no such effect was observed

The patient died nine days after the fourth transfusion Post-mortem examination showed pulmonary oedema, fatty degeneration of the heart, plastic pericarditis, bilateral adhesive pleurisy, hepatomegaly and splena-megaly. There was hypertrophy of the bone marrow

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* NOTE that in this and the subsequent figures the time scale differs to take account of the widely varying rates of destruction

TABLE V

Survival experiments in a case (Case 36) of idiopathic acquired hæmolytic anæmia

Date	Details of transfusions	Hb%	Count of unagglutinated cells per c.mm		
1942 10th Sept	*Patient's blood group A_2 B Rh+* Transfused with 530 c c of a concentrated erythrocyte suspension (group O) in 17 minutes				
	Before transfusion	30	3,000		
	5 min after transfusion	43	940,000		
16th Sept	Six days after		71,000		
22nd Sept	Transfused with 514 c c of a concentrated erythrocyte suspension (group O) in 15 mins				
	Before transfusion		6,000		
	8 min after transfusion		937,000		
24th Sept	2 days later		815,000		
29th Sept	7 days later	42	336,000		
5th Oct	13 days later	34	91,000		
5th Oct	Transfused with 500 c c of a concentrated erythrocyte suspension (group O)				
	Immediately after	48	787,000		
8th Oct	3 days later	47	588,000		
15th Oct	10 days later		125,000		
21st Oct	16 days later		64,000		
21st Oct	Transfused with 495 c c concentrated erythrocyte suspension of group O Rh+blood followed by 535 c c of group A_1 Rh-blood		(1) O Rh+ cells (after agglutination with group O serum)	(2) A_1 Rh- cells (after agglutination with anti Rh serum	(3) O Rh+ and A_1 Rh- cells (after agglutination with group A serum)
	Before transfusion		64,000	76,000	39,000
21st Oct	After transfusion		756,000	882,000	1,677,000
25th Oct	4 days later		532,000	644,000	1,034,000
29th Oct	9 days later		90,000	124,000	182,000

Case 37   Male (W E), aged 63   The patient first became ill in April, 1942, and complained of dyspnœa and weakness of the legs   He had also noticed that he had become more yellow and had lost some weight   There

'was no family history of anæmia or jaundice On admission to hospital in June, 1942, his liver and spleen were found to be enlarged , a blood count showed R B C 1,500,000 c mm , Hb 36%, C I 1 2, reticulocytes 9 2% , serum bilirubin concentration, 4 5 mg per 100 c c A blood film showed occasional nucleated red cells The osmotic fragility was slightly increased There was no autoagglutination of the red cells at room temperature The Wassermann and Kahn reactions were negative The patient was treated with repeated transfusions to which he responded with violent febrile reactions but with some improvement

He was readmitted to hospital on January the 26th, 1943, with an acute exacerbation (R B C 960,000 per c mm) In March, 1943, he was transferred to another hospital and it was at this time that he first came under personal observation A blood examination showed R B C 1,300,000/c mm , Hb 32% , reticulocytes 60% , osmotic fragility, trace of hæmolysis in 0 84% NaCl, 50% hæmolysis in 0 55% NaCl , blood film, well marked microspherocytosis , serum bilirubin concentration 6 0 mg per 100 c c , Schumm's test positive No autoagglutination at 19°C It was noted that samples of whole blood, standing at room temperature, started to hæmolyse within one hour of withdrawal from the patient Physical examination patient pale and slightly jaundiced , spleen enlarged and firm

The patient's blood group was found to be O, Rh positive Many transfusions were given during the following 4 weeks and after four of these the survival rate of the erythrocytes was estimated After the first transfusion, in which 1,500 c c of group O Rh negative blood were transfused, the post-transfusion sample was not obtained until 24 hours after the beginning of the transfusion Although this large quantity of blood had been transfused, only 155,000 donor erythrocytes per c.mm remained in the patient's circulation Following subsequent transfusions therefore, a sample was always obtained within two hours or less of the start of the transfusion and the transfusion itself was always completed within 100 minutes In this way it was possible to make more detailed observations on the fate of the transfused cells

As will be seen from Table VI, the rate of destruction of the transfused erythrocytes was exceedingly rapid For instance, after the transfusion on March 31st when observations were made on several different occasions during the 24 hours following transfusion, it was noted that 45% of the transfused erythrocytes were eliminated within nine hours of transfusion The results of this transfusion are displayed graphically in Fig 5 to show the curvature of the slope of elimination

On April the 21st, 1943, splenectomy was performed Many further transfusions were given and the survival of the erythrocytes was estimated following two of these In each case the rate of elimination was found to be still much increased but was distinctly less than before splenectomy

The patient was discharged from hospital approximately seven weeks after operation and appeared to be improved, although the osmotic fragility of his erythrocytes remained almost unchanged, (² trace of hæmolysis in 0 82% NaCl, 50% lysis in 0 58% NaCl) At this time it was noted that there was autoagglutination of the patient's erythrocytes at room temperature, a phenomenon which had been tested for frequently before but never found

During the following two months he received no treatment but his hæmoglobin rose to 75% He was seen again four months later (November, 1943) and by this time his recovery was more striking He was now doing six days work a week and feeling much stronger Blood count R B C 3,760,000 per c.mm , Hb 72%, Hmt 36 5 , reticulocytes 8% , Van den Bergh, indirect, 2 mg per 100 c c , osmotic fragility still greatly increased (lysis began in 0 75% NaCl)
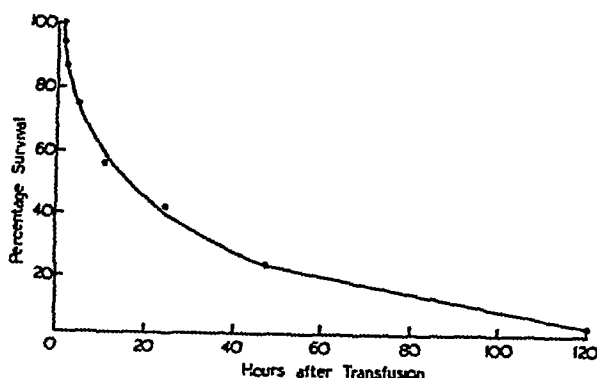
Fig 5 Survival of transfused erythrocytes in a case of idiopathic acquired hæmolytic anæmia (Case 37, second transfusion) , note that the time scale is in hours

The patient was seen once more in February, 1945, and was found to be greatly improved in health He reported that he " never got tired and could walk for miles and miles " A blood examination showed R B C 5,000,000/c mm , Hb 96%, reticulocytes 1% , Van den Bergh 0 36 mg per 100 c c He volunteered to receive a further transfusion and after first being bled of 430 c c he was given 500 c c of a concentrated suspension of group O Rh– erythrocytes Elimination was much slower than it had been previously but was nevertheless far more rapid than normal (complete in about 27 days)

Case 38 Female (E S), aged 45 This patient gave a history of having been ill for many years She was, however, a very hypochondriacal subject and her symptoms were numerous and vague Examination showed that she had a very sallow complexion and an enlarged spleen A blood

TABLE VI

Survival experiments in a case (Case 37) of idiopathic acquired hæmolytic anæmia

Date and time	Time after each transfusion	Details of transfusions	Count of unagglutinated erythrocytes per c.mm
1943 29th March		440 c c of a concentrated erythrocyte suspension (group O Rh−)	
12 15 p m	5 minutes		461,000
3 30 p m	3 hours		232,000
30th March.			
12 0 p m	24 hours		127,000
31st March			
10 25 a.m	48 hours		19,000
10 25 a m — 11 55 a.m		1,000 c c of a concentrated erythrocyte suspension (group O Rh−)	
12 0 p.m	5 minutes		1,198,000
12 30 p m	½ hour		1,139,000
1 0 p m	1 hour		1,040,000
3 40 p m	3¾ hours		894,000
8 50 p m	9 hours		676,000
1st April			
11 30 a m	24 hours		509,000
2nd April			
9 45 a.m	46 hours		282,000
5th April.	5 days		54,000
19th April			20,000
10 55 a m	Before	500 c c of a concentrated erythrocyte suspension (group O Rh−)	
11 18 a m	6 minutes after		646,000
1 55 p m	2¾ hours		533,000
20th April	24 hours		233,000

TABLE VI—(cont)

Date and time	Time after each transfusion	Details of transfusions	Count of unagglutinated erythrocytes per c.mm
21st April		SPLENECTOMY	
26th April		500 c c of a concen trated erythrocyte sus pension (group O Rh–)	
27th April	1 day		880,000
28th April	2 days		784,000
30th April	4 days		470,000
1st May	5 days		378,000
3rd May	7 days		249,000
13th May	17 days		68,000
4th June	Before	500 c c of a concen trated erythrocyte sus pension (group O Rh–)	19,000
	Immediately after		454,000
5th June	1 day		423,000
8th June 1943	4 days		203,000
13th Feb 1945	Before	500 c c of a concen trated erythrocyte sus pension (group O Rh–)	69,000
	½ hour		661,000
	3½ hours		648,000
18th Feb 1945	5 days		477,000
28th Feb 1945	13 days		290,000
12th Mar 1945	27 days		52,000

examination in May, 1942, showed   R B C 1,480,000 per c mm ,   Hb 35% , C I   1 18 , M C V 137 μ^3, reticulocytes 41%   Many nucleated red cells were observed in a film of peripheral blood   Osmotic fragility , faint trace of lysis in 0 76% NaCl, 50% lysis in 0 45% NaCl, 100% lysis in 0 32% NaCl   Serum bilirubin 1 0 mg per 100 c c   There was moderate autoagglutination at refrigerator temperature but none at room temperature

Two transfusions were given and the erythrocytes were eliminated rapidly on both occasions, e g , only about 13% survival was found eleven days after the first transfusion and only about 23% eleven days after the second   After these transfusions the patient left hospital in an improved state , she was seen two months later and found to be still well   Hb 80% ,

reticulocytes 12%, osmotic fragility less than before, *t e*, trace of lysis in 0 52% NaCl, 50% in (less than) 0 42% NaCl

Three months later the patient was readmitted to hospital and found to be worse again, R B C 1,440,000 per c mm, Hb 34%, CI 1 18, Hmt 17 5, M C V 121 μ^3, reticulocytes 42%, osmotic fragility trace hæmolysis in 0 72% NaCl, 50% lysis in 0 46% NaCl   A blood film showed well marked microspherocytosis   It was noted that there was now strong autoagglutination at room temperature but after transfusion this phenomenon disappeared again only to reappear one week later and disappear once more after a further transfusion   Cell survival tests showed that the rate of destruction was slightly higher than it had been previously, for elimination was almost complete by eleven days after transfusion   The estimates made in this survival test are plotted graphically in Fig 6 and the protocols of this and two subsequent survival tests are given in Table VII
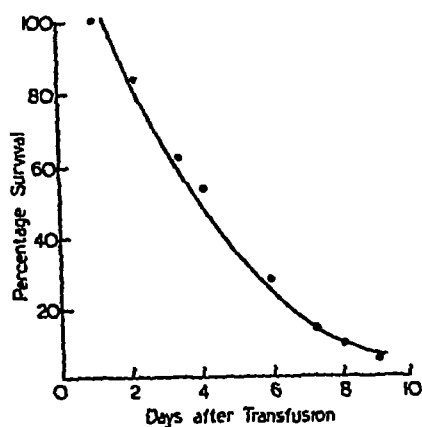
Fig 6
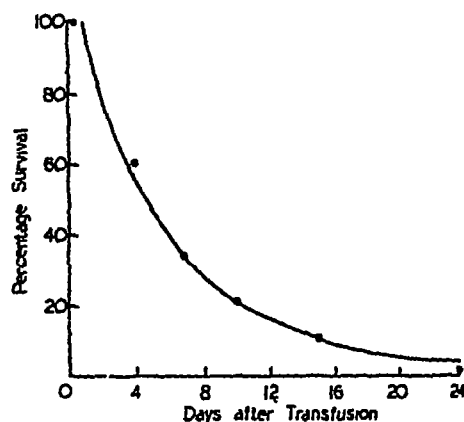
Fig 7

Fig 6   Survival of transfused erythrocytes in a case of idiopathic acquired hæmolytic anæmia (Case 38, fourth transfusion)

Fig 7   Survival of transfused erythrocytes in a case of idiopathic acquired hæmolytic anæmia (Case 39)

Splenectomy was performed on January the 1st, 1943, the equivalent of approximately three litres of blood being transfused before, during and after the operation   Samples of blood taken four days after the operation showed autoagglutination at room temperature once more   A cell survival test was commenced at this time after a further transfusion had been given The rate of elimination was found to be distinctly slower than on any previous occasion and this finding corresponded well with the observation of a low reticulocyte count at this period, namely 3% on January the 4th   Schumm's test was carried out for the first time five days after operation and was weakly positive   Unfortunately the survival of the erythrocytes of this transfusion could not be followed to completion because another transfusion of the same

TABLE VII

Survival experiments in a case (Case 38) of idiopathic acquired hæmolytic anæmia

Date and time	Time after each transfusion	Details of transfusions	Count of unagglutinated erythrocytes per c.mm
15th Dec 1942	Before	1,000 c c of a concen trated erythrocyte sus pension (group O, type N)	10,000
16th Dec 11 30 a.m	24 hours		1,392,000
17th Dec 2 30 p.m	51 hours		1,155,000
18th Dec 6 00 p.m	78 hours		860,000
19th Dec 12 30 p.m	121 hours		748,000
21st Dec 10 0 a m	167 hours		382,000
22nd Dec 6 0 p.m .	199 hours		200,000
23rd Dec 12 30 p.m	217 hours		129,000
24th Dec 11 0 a.m	9 days		84,000
26th Dec	11 days		49,000
30th Dec	15 days		34,000
1st Jan 1943		SPLENECTOMY	
5th Jan	Before	Approx 500 c c of a concentrated erythro cyte suspension (group O, type N)	14,000
6th Jan	1 day		715,000
8th Jan	3 days		550,000
11th Jan	6 days		420,000
13th Jan	8 days		477 000
16th Jan	11 days		320,000
18th Jan	13 days		264,000
19th Jan		1,000 c c blood (group O, type N)	
23rd Jan			962,000
3rd Feb			644,000
13th Feb	25 days		541,000
15th Mar	55 days		59,000

type was given on January the 19th   Twenty-five days after this second transfusion there were found to be approximately 530,000 donor cells per c mm surviving which again indicated that destruction was occurring much less rapidly than before splenectomy   Fifty-five days after this last trans fusion there were no donor erythrocytes remaining in the patient's circulation so that destruction was nevertheless far more rapid than normal Four months after splenectomy the patient was very well and her Hb value was found to be approximately 80%   An estimation of osmotic fragility at this time gave the following results   trace hæmolysis in 0 60% NaCl , 50% lysis in 0 48% NaCl and 100% lysis in 0 42% NaCl

She was seen once more in February, 1945, and appeared to be in good health   The results of a blood examination were as follows   R B C 5,280,000 per c mm , Hb 92% , Hmt 45 0 , reticulocytes less than 0 1% , Van den Bergh 0 16 mg per 100 c c   Fragility still increased (Table VIII)

Case 39   Female, (M C), aged 50   This patient had been attending hospital for many years with indefinite complaints   In December, 1942, she started to become dyspnœic   In January, 1943, physical examination revealed generalised pallor and splenomegaly and a blood examination showed   R B C 1,250,000 per c mm , Hb 40% , C I 1 4 , M C D 7 8μ , reticulocytes 40% , 7 nucleated R B C per 100 W B C   Osmotic fragility, trace hæmolysis in 0 72% NaCl ,   50% lysis in 0 47% NaCl ,   100% in 0 34% NaCl   Serum bilirubin 1 5 mg per 100 c c , Schumm's test positive The blood showed no autoagglutination at room temperature, (fairly strong autoagglutination at 5°C)   Group O, Rh +

The survival of transfused erythrocytes was estimated and found to be rapid, although not so rapid as in Cases 36-38 (Fig 7)   The patient was advised to submit to splenectomy but declined

In May, 1944, she was reported to be well , she exhibited no clinical jaundice but the spleen was just palpable   R B C 5,000,000 per c mm , Hb 102% , M C D 6 8μ

In February, 1945, she was seen once more and now seemed to be completely recovered   She reported that she had had a normal colour for the past two years , her spleen was no longer palpable   The osmotic fragility of her erythrocytes was now normal   Blood was taken from her for experimental transfusion to a suitable recipient (a patient convalescent from gastrectomy), and she was then transfused with blood from normal donors   The survival of normal blood in her circulation was found to be normal for the time for which it was followed, i e , 86% survival 22 days after transfusion   The survival of her erythrocytes in the convalescent recipient was also normal for the duration of the experiment, i e , 54% survival at 57 days after transfusion   These observations provide very strong evidence of a complete recovery from the hæmolytic process

Summary of above four cases   These cases showed not only very rapid elimination of the transfused erythrocytes but also varying degrees of

curvature of the slope of elimination, a phenomenon first described by Brown and others (4)

Two of the four cases underwent splenectomy, two months later both showed considerable clinical improvement with an increase in hæmoglobin levels and a reduced rate of destruction of transfused erythrocytes However, the osmotic fragility of their erythrocytes was unchanged (*see* Table VIII) Two years later both had regained good health and their blood values (including reticulocyte counts) had returned to normal However, the osmotic fragility of their red cells, although less than previously, was still considerably greater than normal and, in the one case in which it was measured, the survival of transfused erythrocytes was still considerably diminished

A third case appeared to recover completely without active treatment

Despite the similarity of these cases with those of familial hæmolytic anæmia, the observation of a rapid elimination of transfused erythrocytes from normal donors compared with a normal rate of elimination in patients with familial hæmolytic anæmia, makes it certain that these are instances of a disease of different ætiology

Since this paper was completed, it has been shown (3) that a distinction between these two diseases can also be made on serological grounds This new evidence, the evidence from the four cases presented here and the results of other previously unpublished work bearing on the distinction between the two types of acholuric jaundice are being summarised elsewhere (13)

2 *A further case of acquired hæmolytic anæmia (idiopathic type)* This case is described separately because there were three features, namely the youth of the patient, the very small change in the osmotic fragility of the erythrocytes and the failure to respond to splenectomy, which distinguished it from the cases in the group described above

Case 40 The patient was a boy aged 14 who first became ill in 1939, when he had a mild attack of jaundice In 1941 he attended hospital again and gave a history of attacks of purpura and epistaxis extending over the previous 18-24 months On examination, his colour was normal, his spleen could not be palpated, a blood examination showed R B C 5,200,000 per c mm, Hb 82%, C I 0 79, platelets 220,000 per c mm, W B C 6,000 per c mm Bleeding time 5 minutes, coagulation time 2 minutes The patient recovered from the attack and remained well until May, 1942, when he had an attack of vomiting and epigastric pain followed two days later by jaundice

He was admitted to Hospital on 6th June, 1942, and found to have generalised jaundice, and an enlarged spleen and severe anæmia A blood examination showed R B C 630,000 per c mm, Hb 15%, Hmt 8%, M C V 127 μ^3, reticulocytes 8 0% A stained film showed some poikilocytosis and anisocytosis, some myelocytes and very numerous nucleated erythrocytes Osmotic fragility very faint trace in 0 55% NaCl, 50%

TABLE VIII

*Osmotic fragilities in four cases of acquired haemolytic anaemia (idiopathic type) **

Percentage haemolysis in different concentrations of saline

Saline concentration (grams per 100 ml)	NORMAL	CASE 36	CASE 37 (Splenectomy on 21st April, 1943)			CASE 38 (Splenectomy on 1st January, 1943)					CASE 39	
			26thMar 1943	8th Jun 1943	14thFeb 1945	18thMay 1942	10thSept 1942	8th Dec 1942	15thMar 1943	8th Feb 1945	4th Mar 1943	16thFeb 1945
0·30	—	—	—	—	—	—	—	—	—	—	100	100—
0·32	—	—	—	—	—	100	—	—	—	100	—	—
0·34	(34—40)	—	100	100—	100—	99+	—	100—	100	—	100	100—
0·36	50%	100	—	—	—	99	99	95+	—	100—	100—	—
0·38	—	—	100	100—	100—	95	90	95	100—	—	100—	80
0·40	—	95	⎱	—	—	90	50—	90	—	80+	—	—
0·42	—	—	—	90	80+	80	10+	80	80	—	—	25
0·44	trace	—	80+	—	—	65	5	50	70	80	60	—
0·46	—	40+	⎰	80+	50	30+	3	30	50	45	45+	trace
0·48	—	—	80	80	—	20	tr	10+	30—	5+	20—	—
0·50	—	30	80+	—	10+	10	ft tr	10	10	—	10—	Nil
0·52	—	—	60	70	—	6	0	8	10	Nil	5+	—
0·54	—	—			trace	—						—

0 56	—	—	—	—	—	—	—	—	—	—	—	—
0 58	—	25—	45	—	ft tr	—	4	—	7	—	trace	5
0 60	—	—	—	50	—	—	—	—	—	—	—	—
0 62	—	15	35	—	—	2	—	5	—	trace	5
0 64	—	—	—	20	Nil	—	—	—	—	Nil	—
0 66	—	—	30—	—	—	tr +	—	—	—	—
0 68	—	7	—	15	—	—	—	—	Nil	—
0 70	—	—	—	—	—	tr	tr +	tr	trace	5—
0 72	—	—	—	7	—	—	—	—	—
0 74	—	tr	—	5—	—	ft tr	—	—	—	Nil
0 76	—	—	—	—	—	—	0	—	—
0 80	—	—	? tr	—	—	—	—	—	—
0 82	—	—	—	? tr	—	—	—	—	—
1 80	—	—	? tr	—	—	—	—	—	—
0 86	—	Nil	—	—	—	—	—	—	—

* All the measurements of osmotic fragility recorded here were made by Drs J V Dacie and Nancy Richardson

hæmolysis in 0 39% NaCl, serum bilirubin concentration 4 mg per 100 c c The patient (group O, type M) received several transfusions and it was noted that the Hb value fell rapidly again on each occasion The survival of the transfused erythrocytes was estimated and it was observed that approximately two-thirds of the erythrocytes were eliminated during the four days following transfusion (*see* Table IX)

Splenectomy was performed on July the 8th, 1942, immediately after a very large transfusion had raised the Hb to 70% A further transfusion was given after the operation and it was found that destruction was still proceeding at about the same rate as before operation The patient died approximately four weeks after the operation Blood examination during these last few weeks showed no change compared with those made prior to splenectomy

TABLE IX

Survival experiments in a case (Case 40) of idiopathic acquired hæmolytic anæmia

Date	Time after transfusions	Details of transfusions	Hb%	Count of unagglutinated erythrocytes/ c mm
1942 3rd July	Before	700 c c of a concentrated erythrocyte suspension (group O, type N)	17	41,000
	Immediately after		44	1,250,000
4th July	1 day		40	1,074,000
7th July	4 days		16	402,000
7th July		1,500 c c of a concentrated erythro cyte suspension (group O, MN)		
8th July		SPLENECTOMY		
		600 c c of a concentrated erythrocyte suspension (ON)+500 c c blood (O MN)		
9th July	1 day		74	1,360,000
11th July	3 days		54	
13th July	5 days	650 c c of a concentrated erythro cyte suspension (O MN)		485,000
16th July	8 days		32	365,000
17th July		1,000 c c of a concentrated erythro cyte suspension (O MN)		
21st July	13 days	1,000 c c of a concentrated erythro cyte suspension (O MN)		129,000

(b)   Symptomatic types

(1)   *Acquired hæmolytic anæmia associated with pregnancy*

Case 41   This patient, a woman of 22, was taken ill on March the 15th, 1942, at the thirtieth week of her first pregnancy   A routine blood examination at the third month of pregnancy had shown   R B C 3,850,000 per c mm , Hb 82%   She remained perfectly fit until ten weeks before the expected date of delivery   Then, however, she was suddenly taken ill with fever (T 104 6°F), aching in the limbs and slight jaundice   The jaundice faded but vomiting developed and then persisted   A week later the patient became very jaundiced and she was admitted to hospital   Blood examination   R B C 1,650,000 per c mm , Hb 34%, leucocytes 10,000   Van den Bergh, direct positive   On the following day a transfusion of 500 c c of group O blood was given   Nevertheless two days later the red cell count was only 1,740,000 per c.mm and the Hb 34%   The urine was found to contain bilirubin   A second transfusion (1,000 c c group O blood) was given immediately   Five days later the red cell count was 2,480,000 per c mm and the Hb 52%   After a further five days no change in the blood picture was found and a third transfusion (1,000 c c group A blood) was given   This raised the patient's erythrocyte count to 3,230,000 per c mm and her Hb value to 70%

The patient then returned home and remained fairly well for the following two weeks   At the end of that time however she was re-admitted to a nursing home with a recurrence of jaundice   The 35th week of pregnancy had now been reached   The results of a blood examination were as follows — R B C 1,420,000 per c mm , Hb 36%, M C V 115μ³, reticulocytes 5 8%, leucocytes 3,800 , Van den Bergh 4 3 mg per 100 c c (biphasic), fragility normal , group A Rh +   There was no autoagglutination at room temperature

During the next three days, two transfusions were given (750 c c group A blood and 500 c c group O concentrated erythrocytes suspension) Nevertheless, immediately after the second of these transfusions the patient's red cell count was only 1,750,000 per c mm and her Hb value 38%

On the following day, Cæsarean section was performed and a healthy female child was delivered   Immediately afterwards a further transfusion of 1,500 c c blood (group A) was given to the mother   Two days after delivery, the red cell count was 2,950,000 per c mm and the Hb 58% Thirteen days later the red cell count had fallen to 2,000,000 per c mm and the Hb to 42%   The erythrocytes transfused on April the 19th were now almost completely eliminated (*see* Table X)   The percentage of reticulo- cytes had risen to 11 4   A further transfusion was given on the following day (1,000 c c of a concentrated erythrocyte suspension, group O)   The Hb was raised to 60% by this transfusion but the value fell to 54% two weeks later and the erythrocytes were eliminated almost as rapidly as before However, the Hb subsequently rose spontaneously and reached a value of

80% after a further six weeks   A normal blood picture was regained within four months

It will be noted that in this case the slope of elimination was approximately linear (*see* Fig 8)
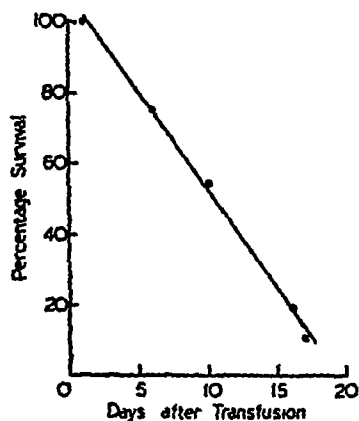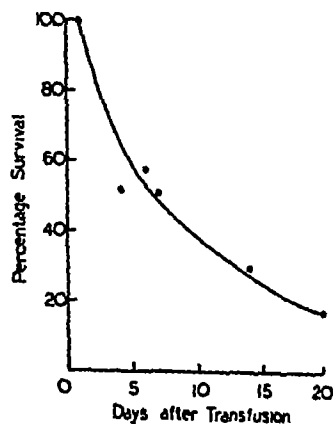
Fig 8   Survival of transfused erythrocytes in a case of acquired hæmolytic anæmia associated with pregnancy (Case 41)

Fig 9   Survival of transfused erythrocytes in a case of acquired hæmolytic anæmia associated with pulmonary tuberculosis (Case 42)

TABLE X

Survival experiments in a case of acquired hæmolytic anæmia associated with pregnancy
(*Case* 41)

Date	Days after transfusion	Details of transfusion	Unagglutinated cells / c mm
1942 19th April	Before	500 c c group O concentrated erythrocyte suspension	10,000
19th April	Immediately after	CAESAREAN SECTION	783,000
20th April	1		820,000
25th April	6		619,000
29th April	10		455,000
5th May	16		174,000
6th May	17		105,000
6th May	900 c c group O concentrated erythrocyte suspension		
8th May	2		1,247,000
20th May	14		167,000
25th May	19		95,000

I am indebted to Dr E ff Creed, who made all the observations in this case, with the exception of the survival tests, and under whose care the patient remained throughout her illness, for permission to report these results

(2) *Hæmolytic anæmia associated with pulmonary tuberculosis*

Case 42   This patient, a woman aged 22, had very advanced bilateral pulmonary tuberculosis   She developed a severe anæmia, but despite repeated transfusions her Hb remained low   A blood examination showed R B C 1,200,000 per c mm , Hb 26%, film, marked polychromasia Osmotic fragility , trace hæmolysis in 0 52% NaCl , 50% in 0 45% NaCl Plasma bilirubin 2 mg per 100 c c   The patient's blood group was A, subgroup A_2, Rh+   The survival of transfused erythrocytes of group O Rh+ was estimated and found to be very much reduced (Fig 9)   It will be noted that the slope of elimination was curved
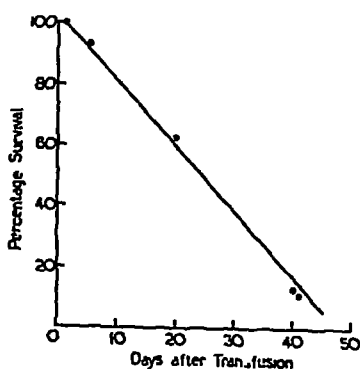
Fig 10
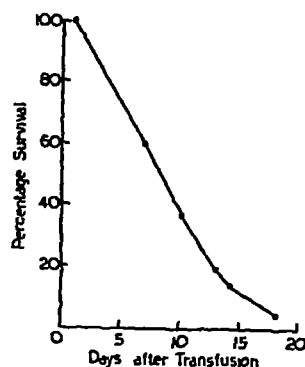
Fig 11

Fig 10   Survival of transfused erythrocytes in a case of acquired hæmolytic anæmia associated with acute osteomyelitis (Case 43)

Fig 11   Survival of transfused erythrocytes in a case of acquired hæmolytic anæmia associated with septicæmia and sulphonamide therapy (Case 44)

(3) *Hæmolytic anæmia associated with severe acute osteomyelitis*

Case 43   The patient was a woman aged 35, who had a past history of a productive cough for twelve years , repeated examinations of the sputum for tubercle bacilli had been negative and on clinical and radiological evidence, bronchiectasis had been diagnosed   In June, 1941, following an attack of maxillary antritis, she developed an empyema of the maxillary antrum and this was soon followed by osteomyelitis of the facial bones   Sulphapyridine (20 grams in five days) was administered but there was no improvement She was found to be anæmic (R B C 2,030,000 per c.mm , Hb 43%) Approximately forty days later she was still anæmic (R B C 1,450,000 per c.mm , Hb 38% , leucocytes 4,400, polymorphs 74%, lymphocytes 22 5%, monocytes 3 5%   The polymorphs were very immature   Two normoblasts were seen in counting 200 leucocytes)

A blood transfusion was given—approximately 700 c c of a concentrated erythrocyte suspension prepared from blood stored for 48 hours in citrate-glucose solution   As seen in Fig 10 elimination was rapid and the slope of elimination approximately linear   However, no deductions about the exact shape of the curve can safely be drawn as there was the following evidence of a change in the rate of destruction   The patient was transfused again fifty days after the first transfusion was given , by this time she was far worse, constantly pyrexial and semi-comatose   There was evidence of a greatly increased rate of elimination, for the count of donor erythrocytes fell from 1,356,000 c mm one day after transfusion to 724,000 c mm seven days after transfusion

The hæmolytic process in this case does not seem likely to have been due to sulphapyridine   Elimination of transfused erythrocytes was rapid during the period between forty and eighty days after the first course of sulphapyridine and the second course of sulphapyridine was only given one day before the survival test was completed

(4)   *Hæmolytic anæmia associated with streptococcal septicæmia and sulphonamide therapy*

Case 44   Following an abortion and a subsequent manual removal of an adherent placenta, the patient, a woman aged 22, developed streptococcal septicæmia despite the administration of sulphanilamide   The course of events was as follows —

22nd March, 1942	Admitted to hospital with a history of persistent uterine bleeding for the past two months
23rd March	Adherent placenta removed manually
24th March to *27th March*	18 grams sulphanilamide administered by mouth, temperature rose to 103°F
27th March	R B C 2,520,000 c mm , Hb 65%, W B C 1,600 c mm , polymorphs 77%, lymphocytes 23%
28th March	Blood transfusion (600 c c , group O)
29th March	Blood culture taken—hæmolytic streptococci grown
31st March	R B C 2,800,000 c mm , Hb 50%, W B C 5,800 c mm , 2 normoblasts per 100 W B C Second blood transfusion given (1,000 c c group O)
3rd April to *13th April*	Total of 55 grams of sulphapyridine administered by mouth
14th April	Transfusion of 1,080 c c group A blood
16th April	R B C 2,590,000 , Hb 57%, reticulocytes 7%, M C V 117 μ^3 , serum contained agglutinin active at 5°C but not at room temperature, osmotic fragility very slightly increased, viz , lysis began in 0 52% NaCl
19th April	Evidence of toxic reaction to sulpha drugs, viz , rash on body and face, œdema of eyelids, albuminuria Further progress, rapid recovery

Estimates of the survival of group O erythrocytes were made from April the 1st onwards , thus the combined totals of the erythrocytes surviving from the transfusions given on March 28th and March the 31st were measured (Fig 11)   For this reason, accurate determination of the

slope of elimination is impossible , however, it appears that the main part of the slope was linear   The terminal curvature of the slope could be explained by the disappearance of the erythrocytes of the first transfusion at this stage or by the progressive improvement of the patient

(5)   *Acute hæmolytic anæmia associated with chronic malaria ("Black-water-fever ")*

Case 45   This patient, a young white adult, returned to England from West Africa in December, 1942   Whilst abroad he had had several attacks of malaria   He took quinine regularly during his journey home and after his arrival in England, but during the Christmas holiday his supply of tablets became exhausted and he developed an attack of malaria   He was admitted to hospital and the administration of quinine was begun again   After 48 hours he developed an acute attack of jaundice and hæmoglobinuria   On examination of a blood film, one subtertian ring was found   Repeated transfusions were given together with large amounts of fluid and alkali by mouth   The patient remained very ill and continued to pass hæmoglobin in his urine for four days   Thereafter recovery was rapid

The following observations were made —

Patient's blood group—A

Date	Days after transfusion of group O blood	Patient's Hb%	Donor erythrocytes c.mm
1943 4th Jan	Day of onset of hæmoglobinuria	64	—
4th Jan	11 p m Transfusion of 1,000 c c group O blood		
5th Jan	11 30 a.m 1	64	739 000
5th Jan	Transfusion of 1,000 c c group	A concentrated	erythrocyte suspension
6th Jan	2	58	633,000
6th Jan	Transfusion of 1,000 c c group	A concentrated	erythrocyte suspension
7th Jan	3	68	
8th Jan	Transfusion of 1,000 c c group	A concentrated	erythrocyte suspension
	4	70	444,000
	Hæmoglobinuria ceased		
13th Jan	9	70	408,000
23rd Jan	19		388,000
3rd Feb	30		218 000

Thus the rate of elimination of transfused erythrocytes was extremely rapid during the period of hæmoglobinuria but thereafter was within the limits of normality   Previous workers (11) from observations of a rapid fall in total red cell count after transfusion, have also reached the conclusion that normal cells, as well as the patient's own cells, are destroyed rapidly in black-water fever

(6) *Acquired hæmolytic anæmia associated with lymphadenoma*

Case 46   This patient, a male aged 20, had had lymphadenomatous glands for the past ten years   During the last few months, observations had suggested that he had developed a hæmolytic anæmia, viz   R B C 2,080,000 ,   Hb 35% ,   reticulocytes 12% ,   osmotic fragility slightly increased   The bone marrow showed an increase in erythroblastic activity   There was an increased excretion of stercobilin in the stools

A very large transfusion was given (2,000 c c of a concentrated erythrocyte suspension)   Rapid elimination of the transfused erythrocytes was observed ,   survival at one day 1,940,000/c mm ,   survival at 30 days, 670,000/c mm   Insufficient estimates were made to decide with certainty the character of the elimination curve

Case of increased rate of elimination of transfused erythrocytes due to Rh incompatability

The usual conception of the fate of transfused erythrocytes after an incompatible transfusion is of a sudden massive destruction with resultant hæmoglobinuria and jaundice   It may not be generally appreciated that the elimination of incompatible erythrocytes may be a gradual affair and that it is always necessary to have incompatibility in mind as a possible cause of an increased rate of elimination   The following case is reported here as an illustration of this point

Case 47   The patient had had three previous normal children, in 1936, 1937, and 1939   At the time of the birth of the third child she suffered an ante-partum hæmorrhage and received a transfusion of blood from two donors   On June the 21st, 1943, she gave birth to a fourth child, which appeared normal   Following this delivery, she had a very severe uterine hæmorrhage   A blood transfusion was begun and, owing to continued hæmorrhage, very large quantities had to be given   Over a period of several hours, nine pint bottles of blood and seven bottles of serum were transfused   It proved impossible to test the blood of all these donors but it was known that one of them was Rh negative   It is, of course, probable that the majority of the rest were Rh positive

A pretransfusion sample of the recipient's blood was obtained, but was not tested until the following morning   It was then found that her erythrocytes were group A, Rh negative and that her serum contained weak anti-Rh agglutinins   In view of this, the infant was carefully observed

It became slightly jaundiced 36 hours after the birth, blood examination showed a hæmoglobin of 118% and only an occasional nucleated red cell in the peripheral blood   The jaundice remained mild and had practically disappeared by the end of a week   The hæmoglobin was then 132% and no nucleated red cells could be found in the peripheral blood   Throughout, the infant received no special treatment and appeared perfectly healthy

Testing of the mother's blood with an anti-Rh serum showed that there was a large proportion of Rh positive cells present   The count of group O cells fell rapidly during the following twelve days (*see* Fig 12), on the eighth day Rh positive cells could still be detected but by the twelfth day there was no longer any trace of them   During the following 5 days there was only a slight fall in the number of surviving donor erythrocytes and it was evident that these were Rh negative cells, some of which, as mentioned above, are known to have been given
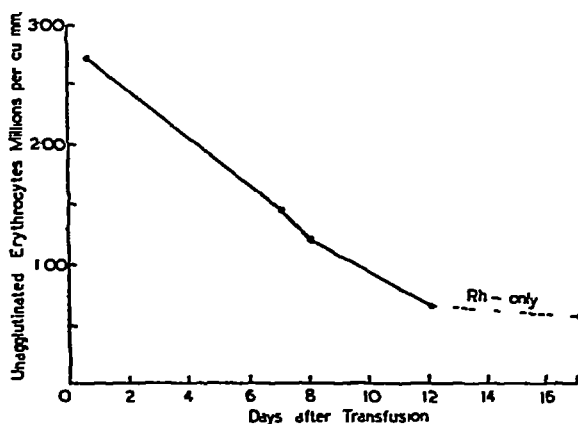
Fig 12   Survival rate of a mixture of Rh+ and Rh— (predominantly Rh+) erythrocytes in the circulation of an Rh— recipient sensitized to the Rh antigen (Case 47)   Note steady rapid elimination of Rh+ erythrocytes, complete by twelfth day after transfusion, thereafter surviving erythrocytes (all Rh—) only slowly eliminated.

From the clinical point of view the only sign suggestive of a hæmolytic reaction was the steady fall in the patient's hæmoglobin value during the two weeks

Wiener (29) has described many instances of this "inapparent hæmolysis" but, so far as is known, no serial quantitative estimates of the rate of disappearance have previously been published

DISCUSSION

It is clear that there are two main kinds of hæmolytic anæmia, (*a*) one in which not only the patient's own erythrocytes but any erythrocytes introduced into his circulation are destroyed at an increased rate, all the cases of acquired hæmolytic anæmia so far investigated have been of this

kind , (b) those patients whose own erythrocytes, due to some defect, congenital or acquired, are more rapidly destroyed than usual, but in whose circulation normal erythrocytes survive for a normal time , this group includes patients with familial hæmolytic anæmia, nocturnal hæmoglobinuria and pernicious anæmia

Dameshek and Schwartz (8) have shown that many of the features of hæmolytic anæmia in man can be closely mimicked in animals by the injection of immune hæmolytic sera   If the acquired human cases are due to the development of some hæmolytic antibody (3), it is clear from the results of the present survival experiments that this antibody is capable of acting not merely upon the patient's own erythrocytes but also on those from normal donors

The difference between the survival rate of transfused normal erythrocytes in familial hæmolytic anæmia, on the one hand, and the four cases of idiopathic acquired hæmolytic anæmia, on the other, is particularly striking   At first sight these latter cases closely resembled cases of familial hæmolytic anæmia, although more careful enquiry showed many of the differences first described by Widal and his co-workers (27), such as the absence of a family history, the increased severity of the patient's illness, etc   Nevertheless confusion between the two conditions may easily occur and a more definite means of differentiation should be of real clinical value   Estimation of the survival time of normal erythrocytes appears to afford one certain means of distinguishing the two conditions from one another

Brown, Hayward, Powell and Witts (4) first called attention to the existence of two types of decay curve of transfused erythrocytes, namely, a linear curve, the most common finding, explicable on the assumption of a constant life of the erythrocyte, subject to little variation , and secondly, a curvilinear slope associated with rapid elimination, seen in a variety of conditions including idiopathic acquired hæmolytic anæmia, and interpreted by them as an exponential curve indicative of the operation of a mechanism destroying red cells independently of their age   These authors also noted the occurrence of occasional cases of an increased rate of destruction without departure from linearity

The observations in the second section of this paper support these findings in that the majority of cases showing rapid destruction of transfused erythrocytes exhibited curves of approximately exponential shape

In view of the difficulties, pointed out in the introduction, of accurate determination of survival figures in the immediate post-transfusion period and of the importance of this period in determining the mathematical nature of any departure from linearity and in view of the possibility of changes in the hæmolytic state of the patient during the period of study (see Case 43), and finally, in view of the small number of observations in most of the cases,

the author has not felt justified in proceeding further with the mathematical analysis of the present results

SUMMARY

(1) Normal survival of transfused erythrocytes has the following characteristics firstly, the number of erythrocytes surviving is inversely proportional to the time since transfusion, and secondly, elimination is complete between the 90th and 130th days

(2) This survival pattern was found when normal erythrocytes were transfused to one normal subject, three healthy convalescent subjects, ten out of eleven patients with varying degrees of anæmia of hypochromic type, five patients with chronic septic conditions, two patients with a carcinoma and one patient with nocturnal hæmoglobinuria

(3) In familial hæmolytic anæmia, in which a normal survival pattern of normal erythrocytes is also found, the donor erythrocytes retain their normal fragility whilst circulating in the patient's blood-stream

(4) By contrast with the finding of a normal survival of normal erythrocytes in the above mentioned conditions, the survival of normal erythrocytes transfused to eleven patients with acquired hæmolytic anæmia was grossly diminished in every instance, elimination sometimes being complete in five or six days In most of these cases, the elimination curves were not linear but were of approximately exponential shape

(5) The differential agglutination method of estimating survival offers a means of distinguishing cases of familial and acquired hæmolytic anæmia

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OLIGURIA AFTER ABORTION

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THE war time studies of Bywaters (1), Darmady (2) and their colleagues have focussed attention on acute renal failure with oliguria, which may occur after limb-crushing injuries or after wounds from high explosive missiles In civilian practice, this complication may supervene in a variety of non-traumatic clinical states not primarily affecting the renal tract, *e g* , mismatched blood transfusions, diabetic coma, concealed accidental haemorrhage (3), prolonged labour (4), abortion (5), pyloric stenosis (6), infective hepatitis (7) and operations on the biliary tract (8)   Due to proved symmetrical renal cortical necrosis, it has been reported with pneumonia, scarlet fever, diphtheria and even apparently in healthy subjects (9)

The meagre publications in renal failure complicating abortion have recently been reviewed by O'Sullivan and Spitzer (5) and so far only nineteen cases have been reported   The history, routine investigations and clinical picture excluded as far as possible the known causes* of oliguria after abortion other than a syndrome attributed to a septic abortion and symmetrical cortical necrosis of the kidneys

The former syndrome, briefly reported by Bratton (10), needs further study   The kidneys in such cases show tubular changes with cellular necrosis and interstitial œdema and polymorphonuclear leucocytic infiltration They appear to correspond closely with the kidneys of soldiers dying with oliguria after severe trauma, associated with sepsis and often with injury to a main blood vessel   Enquiries from colleagues at other hospitals has shown that these cases are not too infrequent and a detailed study of them is needed

Symmetrical cortical necrosis of the kidney can occur with abortion, although it is more common towards the end of pregnancy when it may follow a concealed accidental haemorrhage   Duff and More (11) in 1941 reviewed 71 proved cases of cortical necrosis, 48 associated with pregnancy

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* Incompatible blood transfusions
Hæmoglobinuria from sensitivity to quinine
Renal damage due to acetyl sulphonamide crystals
Acute nephritis
Toxic nephritis due to ingested poisons
Pyelonephritis

and 23 with severe infections Since then, further confirmed cases with pregnancy have been added by O'Sullivan and Spitzer (5) and Donnach and Walker (12) The clinical picture is one of profound oliguria or anuria following abortion or premature labour, with death usually occurring between the fourth and twelfth day, although survival until the 32nd day is reported The patients become increasingly œdematous , headache and vomiting are troublesome symptoms , mental clarity is retained, but the blood urea rises to high levels The very scanty urine may be blood stained at first

There are records of eight patients diagnosed as symmetrical cortical necrosis with pregnancy, and who have recovered, but the diagnosis was substantiated in only one, who underwent decapsulation of the kidney The clinical picture in these cases corresponds closely with those which proved fatal (5, 13, 14, 15, 16, 17, 18)

This paper describes the clinical features and biochemical findings in four cases of acute renal failure after abortion They were admitted over a period of 20 months in a Department dealing with approximately 400 abortions annually These four cases were treated conservatively and all recovered Three of the patients were admitted with incomplete abortions The fourth was diagnosed after admission with vomiting They had oliguria persisting for 6, 10, 13 and 14 days after admission During this time their blood urea rose to 365, 550, 400 and 304 mg per 100 c c respectively They were extremely ill, with troublesome vomiting, but remained clear mentally After a spontaneous diuresis, the clinical and biochemical picture rapidly improved, with complete recovery

Treatment

It was planned to give at least 2,000 c c fluid daily by mouth, per rectum or intravenously 500 c c normal saline was allowed with further amounts of saline to cover quantitatively the loss of gastric juice by vomiting In three out of the four cases, it was necessary to give intravenous fluids either as 5 per cent glucose in water or 5 per cent glucose in normal saline Although this amount of saline caused œdema, this was not thought harmful as the level of potassium in the body fluids might be decreased by dilution Vomiting was not discouraged except by aspiration of gastric contents, as it allowed a loss of potassium and urea from the body Hypertonic solutions were given in two cases only 10 per cent glucose was given intravenously for a short time in Case 1, but there was no doubt that it thrombosed the veins more quickly than a 5 per cent solution Case 1 also received 400 c c 4 7 per cent sodium sulphate before we saw her Glucose and lactose up to 3 oz daily were given by mouth Aneurin 25 mg and nicotinic acid amide 200 mg were added occasionally to the intravenous drip Ascorbic acid, 500 mg was also given at intervals No diuretic effect was noted from these vitamin supplements Sodium bicarbonate 1 g 4-6 times daily was given by mouth, but no intravenous sodium bicarbonate was given

Blood transfusions, using fresh blood, and preferably red cells only, were given after careful cross matching   For preference Rh negative blood was used
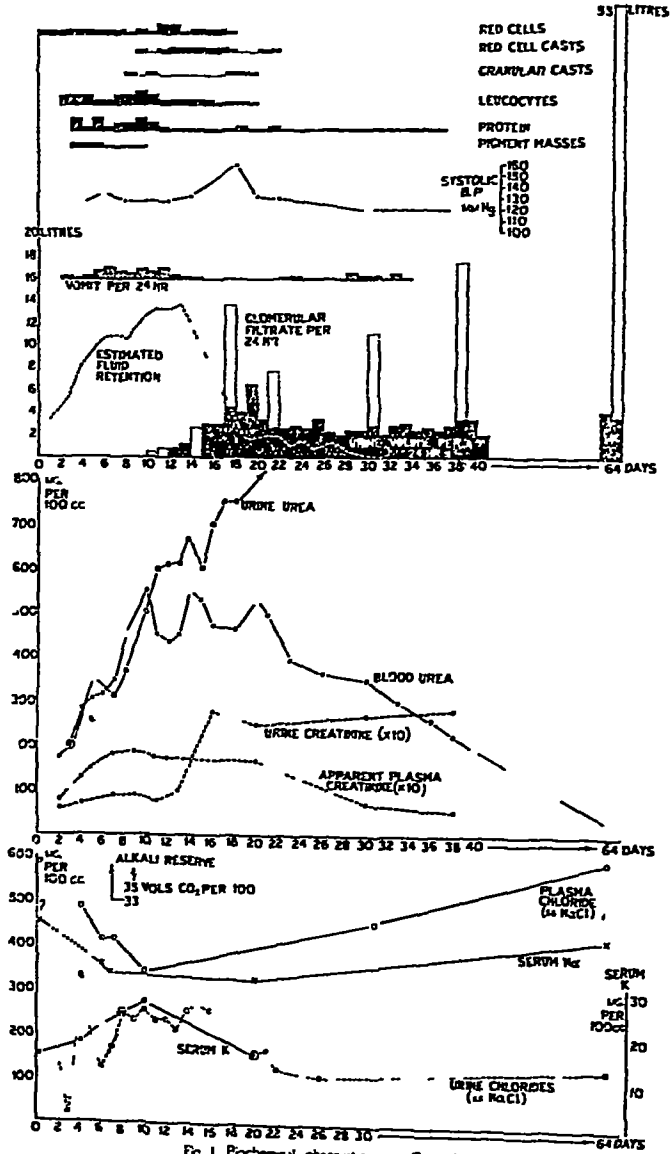

Fig 1 Biochemical observations in Case 1

Penicillin 45,000 units was given by intramuscular injection and repeated five days later   It was found that, owing to the renal failure, blood

penicillin after a single injection stayed at a therapeutic level for five days, and this has been discussed in a previous note by one of us (19)   Apart from a localised pelvic abscess due to *Cl Welchii* in Case 3 the patients so treated were notably free of septic complications, such as skin lesions or bronchopneumonia

<center>BIOCHEMICAL FINDINGS</center>

All biochemical estimations and urine examinations were performed by one of us personally (J H H)

The biochemical findings are set out graphically in Figs 1, 2, 3 and 4 The methods used in estimations were those described by Peters and Van Slyke (20) and by King (21)   In the main, the three cases studied showed very similar changes ,   Case 3 was investigated in greatest detail

The chief interest of the findings lies in the severe loss of renal function which they show, and in the large and abrupt departure from normal electrolyte concentrations which the body will tolerate without gross evidence of further tissue damage, or of mental changes, other than a peculiar listless anxiety   Certain specific findings, however, are worthy of comment

Water metabolism

The patients were too ill to be weighed, but very careful intake and output charts were kept, from which the amount of water retention was calculated   It was assumed that the invisible loss of water by respiration and perspiration was 850 c c per 24 hours   At the height of retention (12-16 litres) all three patients had conspicuous generalised œdema   They drank well throughout and never showed visible perspiration, but were not thirsty

Glomerular filtrate volumes were calculated on the assumption that creatinine is neither excreted nor absorbed by the renal tubules   That the assumption is not strictly justified has been pointed out by Shannon and Smith (22), but the patients were in no condition for inulin clearance tests, and the values calculated for creatinine are probably fair approximations

Urea and creatinine

The figures call for little further comment, except to point out that the serum creatinine was estimated by Folin's alkaline picrate method, which has been shown to estimate chromogenic substances in blood besides creatinine   Allinson (23) used a specific enzyme to destroy creatinine, and obtained values in normal sera for true creatinine, approximately 70% of those for apparent creatinine   In calculating the glomerular filtrate volume, the assumption was made that the true serum creatinine values were 2/3 of the apparent values—a correction which would be consistent in Case 1 with the supposition that the urine was a glomerular filtrate
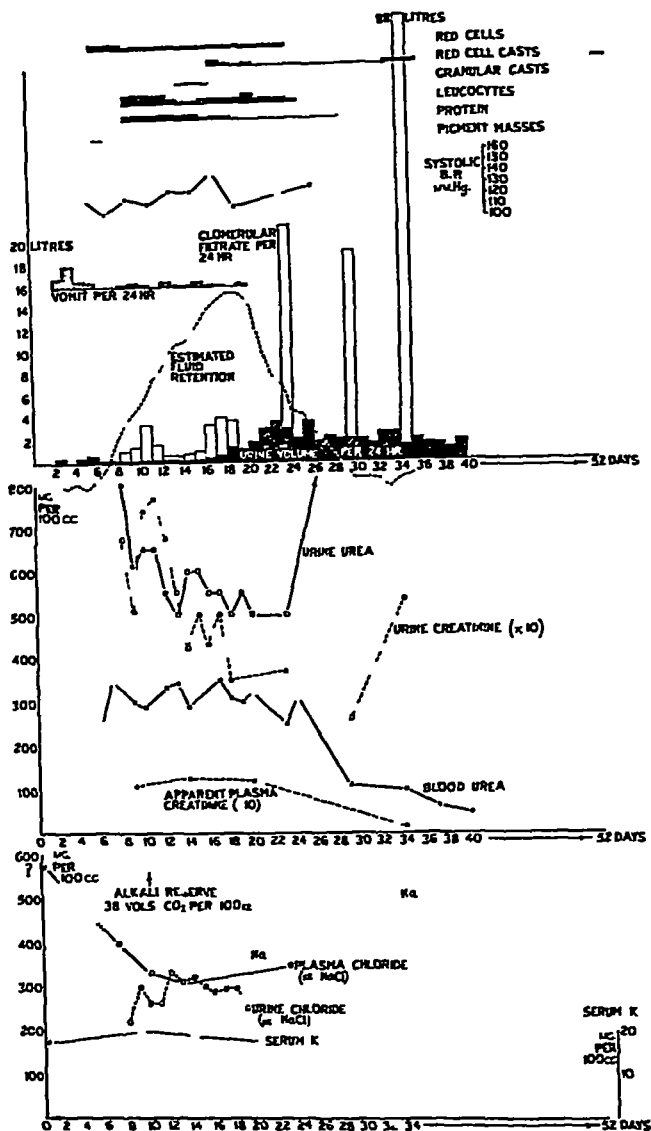
Fig 2. Biochemical observations in Case 2

The variations in the rate of increase and in the final blood urea levels attained may be explained partly by the observation that urea is excreted in the vomit, as well as in sweat and urine, and that the amount of vomiting during the early stages varied from patient to patient

In Case 1, the urine for fourteen days was an almost unadulterated glomerular filtrate, and in Cases 2 and 3 there was evidence of only 2-4 fold
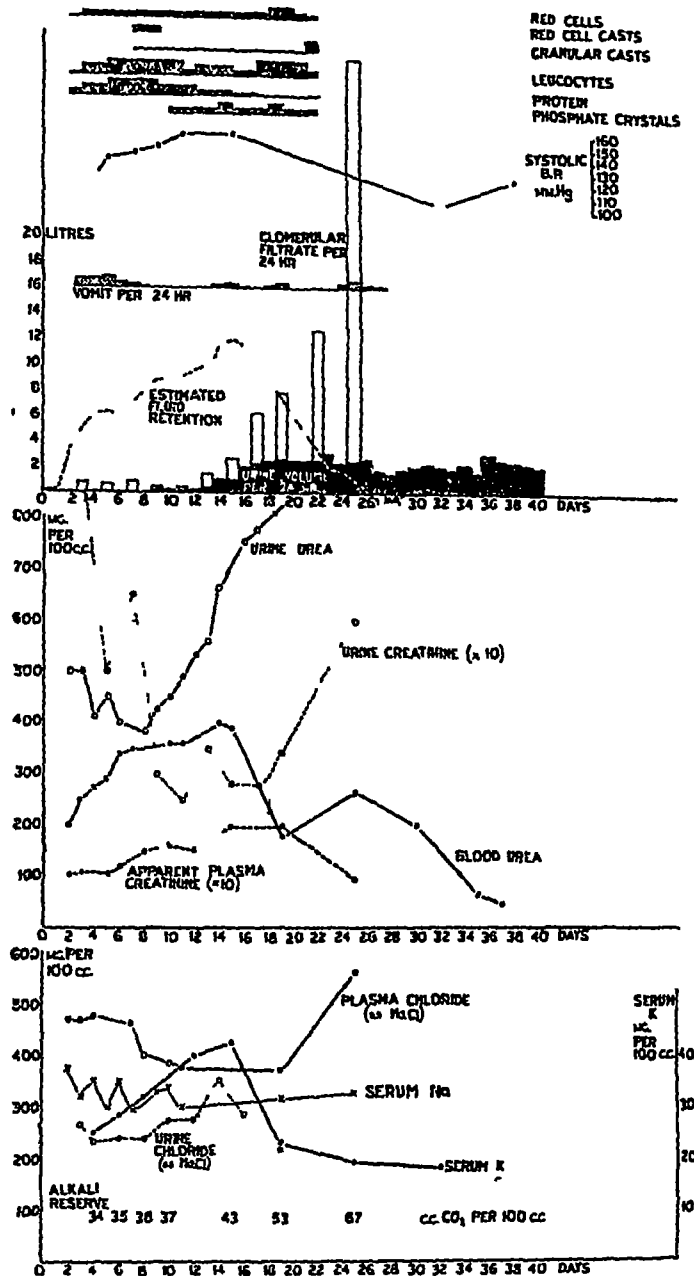
Fig 3 Biochemical observations in Case 3

concentration by re-absorption of water, for periods of twelve and ten days respectively

Chloride

The plasma chloride levels fell very low (normal values 560-620 mg NaCl per 100 c c), and yet the kidneys failed to retain chlorides In addition to the dilution of the body fluids by retained water, chloride loss in the

copious vomit of each patient must have been considerable   In Case 3, on the only occasion when the free HCl in gastric juice was estimated, it was present in concentration 0 08 per cent at a time when the plasma chlorides were 475 mg NaCl per 100 c c   Loss in sweat and urine were relatively insignificant

It is apparent from the curves that loss and partial restoration of power to retain chlorides and urea did not coincide in time, supporting the conclusion which others have made, that the mechanisms for dealing with the two substances are not identical

Potassium

Although three patients showed a rise in serum K to levels of 20 5, 27 5 and 42 5 mg per 100 c c , only in Case 3 was the rise sufficient to cause alarm   Potassium liberated by endogenous cellular breakdown was in part diluted by retained water, but Case 3 (alone of the four) was given drinks flavoured with synthetic flavourings instead of plain lactose and saline Analysis of these fruit drinks, diluted as for use, revealed a potassium content of 8-11 mg K per 100 c c   Their use was stopped immediately, but not before a remarkable electrocardiographic picture had been obtained at a time when the serum K was 40 0 and the Ca concentration 6 0 mg per 100 c c (Fig 5)

Three analyses for K in neutralised vomit showed values only slightly lower than the serum levels at the time, and vomiting may be of considerable importance as a means of eliminating potassium from the body when the kidney fails

Sodium

Serum sodium levels varied less than those of other electrolytes   The values tended to be high (normal 325-350 mg per 100 c c)

Tentative calculations of serum sodium on admission, after allowing for salts administered, were made on the assumption that sodium was distributed throughout the extracellular water   They also are high

Calcium and phosphorus

These were only studied fully in Case 3   As inorganic phosphorus values rose, those for calcium fell, reaching the low level of 5 8 mg per 100 c c   Single high values for inorganic P were found in Case 2, and all three cases were presumably similar   Since no phosphate was ingested during the period of oliguria, the raised values must have been solely due to endogenous liberation

Despite the low Ca values, none of the patients showed signs of tetany nor of latent tetany   In view of the fact that serum proteins were not less than 5 0 per cent throughout, the protein-bound Ca was not necessarily reduced in proportion

Plasma bicarbonate

All estimations of the alkali reserve made during the period of oliguria (the earliest being four days after onset) showed marked depletion, and yet, except for transient episodes in Case 3, there was no marked hyperpnœa Even these transient episodes may have been due rather to the gross anæmia, and it seems likely that the pH of the blood was not much altered from normal

Inorganic sulphur

In Case 3, inorganic sulphate sulphur was estimated by Hubbard's colorimetric method (20) after two or three-fold dilution of the serum to avoid precipitation of benzidine by excess of phosphate   The highest value reached, 35 mg per 100 c c , is notable   Inorganic sulphur is not known to be stored nor metabolised in the body, and under the conditions of Case 3, it must have been derived from endogenous protein breakdown only Between the third and fifteenth days, the plasma values rose steadily from
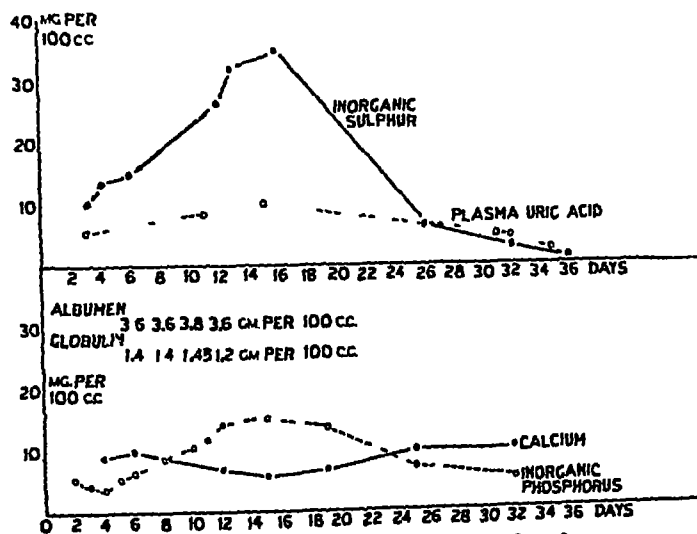
Fig 4   Biochemical observations in Case 3.

10 to 35 mg per 100 c c   Assuming that sulphate was distributed throughout the extra-cellular fluid only (calculated initially as 25 per cent of body weight), and assuming that all retained fluid was extra-cellular, the net production of inorganic sulphur was calculated to be 7 0 g , i e , 0 57 g per diem   Human body proteins generally contain approximately 1 per cent of sulphur, and the endogenous protein breakdown must have been at least 57 g per diem

It is interesting to compare this figure for endogenous protein breakdown with that derived from the values for blood urea in Case 1, taken over the period two to ten days after abortion, when the blood urea rose steadily from 175 to 550 mg per 100 c c   In this case the assumption is made that urea was distributed throughout all body water (calculated initially as 70 per cent of body weight), and the net urea production was approximately 23 5 g per diem   This corresponds to approximately 67 g protein per diem

Uric acid

The values in Case 3 were not particularly high although they reached three times normal (Fig 4) The rate of endogenous production of uric acid is 0 3-0 4 g per diem in adults (24) On the assumption that uric acid is freely diffusible throughout the body water it can be calculated that the uric acid concentration in the blood should increase by approximately 1 mg per 100 c c per diem The observed rate was only 1/3rd of this Loss in the vomit was not investigated

Acid-base balance

Only for Case 3 were sufficient data accumulated to allow calculation of the electrolyte balance The results are set out below, expressed as mille-equivalents per litre
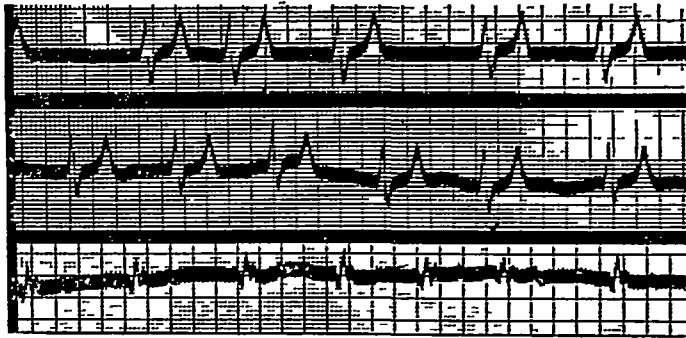
Fig 5 Electrocardiogram Case 3 taken when Serum K was 40 mg per 100 cc and Serum Ca 6 0 mg per 100 cc.

Case 3 *(Mille equivalents per litre)*

DAYS AFTER ABORTION	Na+K+Ca '	' Cl+HCO₃+PO₄ +SO₄+Prot	DIFFERENCE
2	180	108	72
3	154	107 5	46 5
4	140	108 5	31 5
6	139	91 5	47 5
8	143	105	38
10	161	107 5	53 5
11	163	107	56
19	150	110	40
25	164	136 5	27 5 '

In working out the sum of the anions, use was made of the formula for plasma at pH 7 4 (20)

$$\text{Anions (mille equiv per l)} = Cl + 1\,8 \times PO_4 + 2 \times SO_4$$
$$\text{(Mille equiv per l)}$$
$$+ 0\,28 \times alb + 0\,193 \times glob$$
$$\text{(g per 100 c c)}$$

The fluctuation in the difference between the sums of the cations and the anions is mainly due to the fluctuation in the sodium concentration Nevertheless, there is an abnormally large deficit of anions during the period of oliguria and of early recovery, which must be accounted for by acids other than those estimated Negative Rothera and ferric chloride tests were obtained in Case 3 on two occasions during the period of oliguria We are left therefore, without any indication of the nature of the acids involved

Urine

The appearance of cells and casts in the urine is shown for each case separately on the charts The similarity between them is striking Red cells and leucocytes, in number sufficient to make the urine cloudy but not smoky appeared from the onset, before the urine became heavily laden with bacteria, as were all later samples Next came scanty pigment masses (in Cases 1 and 2 only), and then, just before the urine flow began to increase, came scanty granular casts, followed closely by red cell casts Although the urine during the period of oliguria contained up to 1 per cent protein, the protein content was very greatly diminished simultaneously with the re-establishment of urine flow

Serum and urine from Cases 1 and 2 were examined spectroscopically by Dr E G Bywaters, who did not report any abnormal pigment present

DISCUSSION

Incompatible blood transfusion was ruled out by careful checking in Cases 1, 2 and 3 Since all four patients were Rhesus positive, since three of them had had no previous pregnancies, and since none had had previous transfusions, it is most improbable that Rhesus incompatibility was involved

Renal damage due to acetyl sulphonamide crystals was a possibility in Case 2 only, but cystoscopy did not reveal any crystals causing ureteric blockage

Abortifacients were denied in Case 4, stilboestrol had been taken in Case 1 and the remaining two had used douching only No quinine had been taken

Acute nephritis, and pyelonephritis, were improbable causes, judging by the clinical condition and history of the illness

Cl Welchii was isolated from Case 3, but the infection appears to have been localised The amount of antitoxin in the serum was so small (Appendix, Case 3) as almost to preclude significant absorption of Cl Welchii toxin

Antibodies against homologous kidney extracts have been prepared experimentally in rats by Cavelti (25), using streptococci as adjuvants, and the appearance of antibodies was accompanied by nephritis We tested the sera of patient 1 and 2 taken early during the period of oliguria

and during convalescence for antibodies against a 20 per cent suspension of fresh human kidney   Although we used a delicate complement fixation technique, no specific antibodies were detected

The biochemical findings were consistent enough to be used as an indication of the pathological processes at work in the kidney   From the biochemical data, it is obvious that glomerular and tubular function were equally, and almost wholly, suppressed, and that there was no rise in intraglomerular pressure   The temporary nature of the proteinuria and the transient passage only of tubular cell casts suggest that there was no special toxic effect on the tubule, such as poisoning with heavy metal salts may produce   It also suggests that anoxia, if it occurred, was not sufficient in degree to cause any considerable immediate degree of cell necrosis   The whole picture is much more consistent with a temporary great reduction in flow of blood through the afferent arterioles, so that the intraglomerular pressure was too low to permit glomerular filtration, but that sufficient oxygen was available to prevent autolysis of the tubular and glomerular cells   This reduction seems to be most readily explained by spasm with or without thrombosis of the afferent small arteries and arterioles   Prolonged spasm would undoubtedly lead to thrombosis, and the re-establishment of urine flow would require the gradual re-canalisation of the thrombosed vessels, as well as relaxation of residual spasm

The underlying pathological process is uncertain, but the most probable is that whose end-result is found in persons dying with symmetrical renal cortical necrosis   Although the ætiology of this condition is not proven, a clue may be found in the work of Barclay, Trueta, Daniel, Franklin and Pritchard (26), who studied the effect of a tourniquet applied to the intact hind limb of rabbits   They have demonstrated a narrowing of the renal artery, greatly reduced renal cortical blood flow and a shunt of blood through the vessels in the medulla   Similar effects are claimed to be produced by " appropriate nerve stimulation," and the possibility appears of a conceptual unity of the renal complications of hæmorrhage, trauma and abortion   Direct arterio-venous shunts in the renal cortico-medullary zone have been demonstrated by Shonyo and Mann (27) in rats, guinea pigs, dogs, cats and chickens and the requisite anatomical pattern thus appears to be widely found   Prolonged cortical ischæmia of this type, if it occurs in man, as the phenomena of reflex anuria suggests it may, might well cause oliguria and lead to thrombosis in the afferent glomerular arterioles and subsequent cortical necrosis   In association with septicæmia, death might result before thrombosis had occurred, and the histological picture described by Brattan (10) be produced   Even assuming, however, that this is the physiological mechanism, the trigger factors in these cases remain obscure   None had unduly severe circulatory collapse, though all had lost at least 1,500 c c of blood   The degree of blood loss, together with fear and pain in a particularly susceptible individual might be sufficient to set off such a reflex vascular constriction and cause partial renal anoxia

SUMMARY

Four cases of extreme oliguria after abortion are described Oliguria persisted from ten to fourteen days, and the blood urea rose to 350-550 mg per 100 c c and fell to normal after a diuresis had occurred spontaneously The patients were treated conservatively and all recovered

Detailed biochemical observations have been made, from which the glomerular filtration rate and the endogenous protein breakdown have been calculated The very marked deviations from normal electrolyte content of the blood are discussed

Serological examinations gave no clue to the ætiology of the condition

APPENDIX

Case reports

Case 1 (M 5933) Aged 31 Single

Admitted with incomplete abortion at the sixteenth week She had had 27 ergoapiol tablets at the time of the second missed period and an unknown amount of stilboestrol, after the third missed period On admission, she was very pale, the hæmoglobin was 5 2 g per 100 c c A blood transfusion was given and the uterus was then evacuated under general anæsthesia, using pentothal and cyclopropane There was no urine in the bladder twelve hours after admission

The following day, only 45 c c of blood stained urine was obtained The oliguria persisted for ten days and during this time only 262 c c were secreted She had troublesome vomiting, bringing up small quantities at a time, and intravenous fluids were given Her hæmoglobin had fallen to 4 4 g per 100 c c after seven days and 700 c c concentrated red blood cells were given After ten days, her general condition was extremely poor, she was markedly œdematous, the respirations were a little deepened and the pulse was regular Blood pressure was 125/60 mm Hg The gums were bleeding and epistaxis had occurred The blood urea was 550 mg per 100 c c, but her mind remained unclouded On the eleventh day she passed 180 c c urine and on the following days 400 c c, 885 c c, 1,155 c c, 2,670 c c and reached a peak of 6,540 c c five days later Her general condition rapidly improved, the œdema cleared, but her hæmoglobin remained low, and a further 1,080 c c blood transfusion was given The blood urea returned to normal (Fig 1) but the urea clearance was only 30 per cent on leaving hospital

Two years later her general health was excellent, periods regular and there was no nycturia Blood pressure 130/70 mm Hg No proteinuria Blood urea 37 mg per 100 c c Urea clearance test 74 per cent An intravenous pyelogram failed to show any concentration of dye in the pelvis of the kidney

Case 2 (M 6761) Aged 37 Single

Admitted with a sixteenth week abortion She had been douching herself for two months but had not taken any abortifacient Thirty six hours previously she had had severe pain, followed by heavy bleeding and the fœtus was passed On admission, she appeared ill and there were foul smelling pieces of placenta in the vagina The hæmoglobin was 10 6 g per 100 c c blood pressure 90/45 mm Hg Evacuation of the uterus was performed under gas oxygen and pentothal anæsthesia

During the next few days, she had troublesome vomiting and oliguria was noted This persisted for fourteen days, and only 2,360 c c urine were secreted during this time She had been given 20 g of sulphathiozole after admission and to exclude blockage due to crystals, cystoscopy and ureteric catherisation were performed, but no crystals were present She was extremely ill with persistent vomiting and a troublesome hiccough, and intravenous fluids were given She remained mentally clear, but rather drowsy A blood transfusion of 540 c c was given on the ninth day when her hæmoglobin had fallen to 7 0 g per 100 c c By the fourteenth day she was very œdematous, the pulse was regular, blood pressure was 125/80 mm Hg, hæmoglobin 7 0 g per 100 c c, blood urea 304 mg per c c A diuresis then occurred and she passed 480 c c, 1,860 c c, 1,740 c c and 2,040 c c on successive days Her œdema subsided her general condition improved, but her hæmoglobin remained low and she was given 600 c c

concentrated red blood cells two weeks later   Her subsequent progress was entirely satisfactory and her blood urea fell to 40 mg per 100 c c

Twentv two months later, her health was good, periods regular and there was no nycturia Blood pressure was 120/70 mm Hg   no proteinuria, blood urea 40 mg per 100 c c   urea clearance test 55 per cent of average normal

Case 3   (O 3041)   Married   Aged 24

One child aged 3¼   Admitted with incomplete abortion at the sixteenth week   She had been douching herself with soap and Dettol for two months but had not taken any abortifacients Bleeding began the night before admission and she had lower abdominal colic with continuous vomiting, and the foetus was passed before admission.

On examination, she was very pale, still losing her vaginam and the right lower quadrant of the abdomen was tender and rigid   She was given 1,080 c c blood, and the uterus was evacuated under general anæsthesia six hours later   No urine was passed on the day of admission and only 150 c c the following day   The oliguria persisted for thirteen days and during this time she secreted only 1,350 c c of urine   Her blood urea rose to 400 mg per 100 c c She had troublesome vomiting bringing up small quantities at a time   She was given 2,000 c·c fluid daily by mouth as lactose fruit drinks, and also 1,000 c c 5 per cent dextrose saline at intervals to cover the loss of sodium by vomiting   By the thirteenth day she was moderately œdematous, very pale and her hæmoglobin was only 5 5 g per 100 c c   Her breathing had increased in rate and depth   Her pulse had become irregular on the previous day , blood pressure was 160/65 mm Hg   An electrocardiogram showed absent P waves and extremely large T waves in Leads I and II (Fig 5)   The blood urea was 400 mg per 100 c c , serum calcium 6 0 mg per 100 c c   but there was no latent tetany   serum potassium 42 5 mg per 100 c c

In view of the anæmia and her extremely ill condition, she was given a further blood transfusion of 540 c c followed by 2 000 c c 5 per cent dextrose saline and 10 c c 10 per cent calcium gluconate   The same day a diuresis began, and she passed 435 c c and 1,500 c c the next day and, within a few days, she was passing 2 700 c c daily   In spite of the diuresis, her general condition remained extremely poor   her abdomen was rather distended and there was a tender mass in the right iliac fossa   She had a low grade fever and diarrhœa and her hæmoglobin fell again and further transfusions were given   After three weeks, a colpotomy was performed and a large amount of stinking pus was drained   *Cl Welchii* was isolated from the discharge

From then onwards her condition rapidly improved   her hæmoglobin rose to 10 0 g per 100 c c   her blood urea fell to 47 mg per 100 c c and she was discharged home   Three months later her blood urea was 42 mg per 100 c c , her urea clearance test 56 per cent of normal and there was no proteinuria   An intravenous pyelogram showed very poor concentration by either kidney   The outline of the calyces on the right side was just visible after 30 minutes, and they appeared normal

Blood serum taken two weeks after discharge was kindly assayed at the Lister Institute of Preventive Medicine for antibody content to *Cl Welchii*, α — toxin (α — lecithinase)   The serum contained less than 0 01 egg units of antitoxin

Case 4   (N 1370)   Single   Aged 31

Admitted after eight days vomiting and sent into hospital as blood had been seen in the vomitus   She had been losing heavily for one week but she had not missed a period   Her general condition was extremely poor , hæmoglobin 7 7 g per 100 c c   blood urea 285 mg per 100 c c   On examination, she was found to have an incomplete abortion and the uterus was evacuated under cyclopropane anæsthesia   The uterus corresponded with an eight weeks pregnancy   Oliguria was noted and she passed 450 c c , 160 c c   560 c c , 520 c c in spite of a fluid intake of over 2 500 c c daily   On the sixth day after admission (thirteen days after the onset of the bleeding) she passed 1,560 c c of urine, and thereafter had an excellent diuresis It was not clear how much urine she had been passing before admission   The blood urea rose to 365 mg per 100 c c on the sixth day   blood pressure 130/60 mm Hg and she was very apathetic and pale (hæmoglobin 7 0 g per 100 c c)   The vomiting had persisted and she also had hiccough and a troublesome moist cough   There was pitting œdema of the legs   After the diuresis her blood urea fell to normal within two weeks   She denied taking any abortifacients and indeed had not realised she was pregnant

When seen eighteen months later, her health was good, periods regular, no nycturia, blood pressure 120/70 mm Hg , blood urea 36 mg per 100 c c , urea clearance test 75 per cent of normal   There was no proteinuria

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EFFECTS OF OXYGEN, VENESECTION AND DIGITALIS IN CHRONIC HEART FAILURE FROM DISEASE OF THE LUNGS

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PREVIOUS studies on cases of congestive heart failure due to hypertensive, ischæmic and valvular heart disease showed that the resting cardiac output was reduced when the venous filling pressure was conspicuously increased above normal   It was further shown that cardiac output increased when the initial high venous filling pressure was lowered by intravenous digoxin (8) or by venesection (5)   The present paper reports observations on cardiac output and filling pressure in cases of congestive heart failure associated with emphysema and other chronic diseases of the lungs

Methods and material

Right auricular pressure and cardiac output were measured by cardiac catheterisation (7)   Mean right ventricular pressure measured manometrically by catheterisation is expressed as cm of saline above the right auricular pressure   The normal mean right ventricular pressure, measured in the same way, is 10 to 15 cm   The observations were made with the patient's trunk elevated 45°   The normal right auricular pressure is about —7 cm (sternal angle = 0) in this position, but in cases of emphysema without heart failure we have usually found the pressure to be about —10 cm   due perhaps to alteration of the position of the heart in relation to the sternal angle   Arterial samples were obtained from the brachial or femoral artery   Auricular samples were taken immediately after each arterial sample, since some cases showed spontaneous variation of arterial oxygen saturation   Venesection was performed in 5 to 10 minutes and the data given in Table III were obtained immediately after venesection was finished   Intravenous digoxin was given in 1 5 mg doses and the results observed for half to three-quarters of an hour   Clinical material is shown in the appendix

The cases consisted for the most part of patients with chronic bronchitis and emphysema Patients present similar circulatory sequelæ with silicosis, pulmonary carcinomatosis (4) and kyphosis (2), and one example of each is included

TABLE I

Case	Ref No	Age and Sex		Arterial oxygen saturation %	Right auricular pressure cm saline above sternal angle	Cardiac output L per min	Heart rate	Blood pressure mm Hg	Mean right ventricular pressure cm saline above auricular pressure
					GROUP A				
1	5 44	M	69	33	+19	6 9	110	115/50	—
2	8 161	M	31	66	+16	8 4	100	140/92	40
3	8 123	M	42	42	+ 5	6 55	104	118/69	—
4	8 131	M	52	67 5	+ 6	6 6	104	156/42	—
5a	8 143	F	54	61	+14 5	9 65	128	118/?	—
6	6 9	M	56	67	+ 2 5	6 85	92	132/85	—
7	5 27	M	61	76	+ 5	6 46	104	118/95	—
8	8 159	F	30	72	— 3 5	6 05	90	138/86	37
9	8 79	M	56	48 5	+ 8 5	9 0	102	98/60	24
10	9 59	M	30	70	+ 5 5	9 2	114	132/70	32
11	9 0	M	55	68	+ 1 5	10 0	94	160/70	30
					GROUP B				
12	6 131	M	48	59	+14 5	3 5	104	56/?	—
13	9 25	M	44	55	+11	3 7	90	66/46	21
14	5 24	M	60	66	+ 9 5	3 4	120	52/35	—
15	7 143	M	51	61	+13	4 6	104	60/?	—
5b	7 81	F	54	71	+ 1	5 0	108	85/60	—
16	6 11	F	55	46	+ 9 0	4 1	120	50/?	—
17	9 67	M	66	77	+ 8 5	3 8	88	76/?	23

Results

Initial data are shown in Table I Cases are divided into two groups, (A) with systolic blood pressure greater than 90 mm Hg, (B) with systolic blood pressure less than 90 mm Hg Arterial saturation was recorded as low as 33 per cent in one case and below 50 per cent in 3 cases, without loss of consciousness or, in 2 of these cases, a fall in blood pressure Right auricular pressure was increased in all cases, in some to high levels In group (A) cardiac output was increased, in group (B) it was decreased or normal The heart rate was usually moderately increased Mean right ventricular pressure was increased in the cases in which it was measured
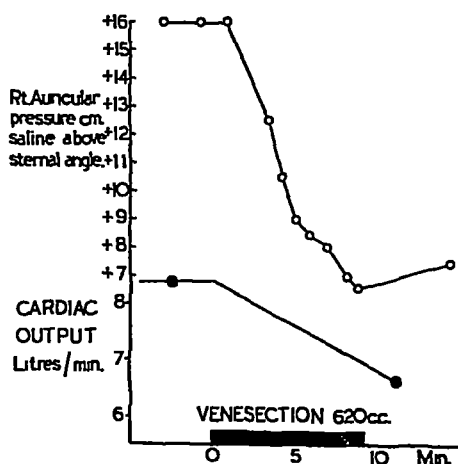
Fig 1 *Case 2* Bleeding causes a fall in right auricular pressure and a fall in cardiac output
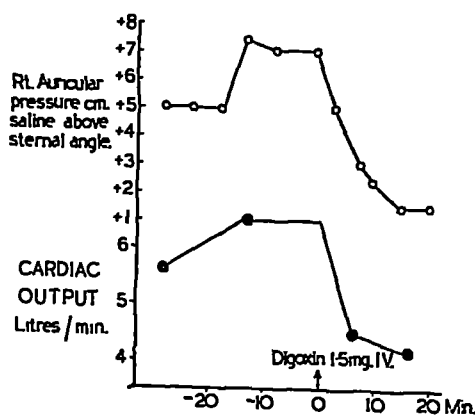
Fig 2 *Case 7* Intravenous digoxin causes a fall in right auricular pressure and a fall in cardiac output

TABLE II

Case No	Time	Arterial oxygen Saturation %	Right auricular pressure (cm saline)	Cardiac output (L per min)	Heart rate	Blood pressure, mm Hg	
Effects of oxygen administration							
8	Before	72	− 3 5	6 05	90	138/86	
	After	96	− 4 5	5 3			
9	Before	48 5	+ 8 5	9 0	102	104/65	
	After	73	+12	7 9	104	118/65	
10	Before	70	+ 5 5	9 2	114	132/70	
	After	92	+ 4 0	8 0	112	132/85	
13	Before	55	+11	3 7	90	66/46	
	After	78	+10	3 9	84	76/50	
15	Before	61	+13	4 6	104	80/?	
	After	78 5	+ 9 5	4 0	104	90/?	
5b	Before	71	+ 1	5 0	108	85/60	
	After	93	0	4 1	116	130/?	
			TABLE III				Amount bled
Effects of venesection							
2	Before	66	+16	8 4	100	140/92	620
	After	71	+ 6 5	6 7	100	142/94	
3a	Before	42	+ 5	6 55	104	118/68	420
	After	52	− 2 5	5 2	114	106/62	
3b	Before	76 5	+ 5 5	6 5	108	140/65	750
	After	68	− 4 0	8 35	106	125/65	
4	Before	67 5	+ 6	6 6	104	156/92	600
	After	68	− 2 5	5 85	94	146/88	
			TABLE IV				
Effects of 1 5 digoxin I V							
6	Before	67	+ 2 5	6 85	92	126/70	
	After	67	− 1 0	6 0	92	132/85	
7	Before	76	+ 5	6 46	104	118/95	
	After	78	+ 1 5	4 2	100	155/95	
9	Before	73	+12	7 9	102	104/65 in O₂ tent	
	After	75 5	+ 5	7 0	104	118/65	
13	Before	78	+10	3 9	84	76/50 in O₂ tent	
	After	65	+ 6 5	6 85	88	108/66	
17	Before	77	+ 8 5	3 85	88	76/?	
	After	73	0	5 2	84	110/?	

Effects of oxygen administration (Table II) Arterial oxygen saturation
increased in every case, in 3 to normal levels Right auricular pressure
showed no constant change, rising in 2 cases, falling in 2, and showing little
change in the other 2 Cardiac output decreased in all except one case
Blood pressure increased

Effects of venesection Four observations in 3 cases are shown in
Table III, and an example in Fig 1 Arterial oxygen saturation showed a
small increase in 2 and a decrease in one observation These changes are
probably not the result of venesection but of spontaneous fluctuations
Right auricular pressure fell in every case Cardiac output decreased in
3 of the observations In Case 3, cardiac output increased when the
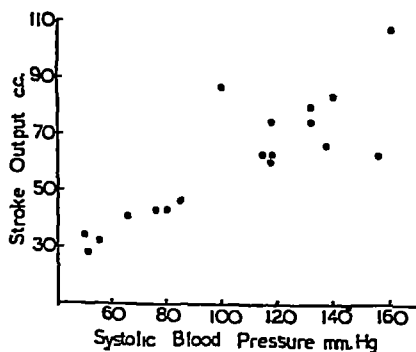
Fig 3 This chart shows relationship between systolic blood pressure and stroke output

procedure was repeated three weeks later During this period the patient
had been severely ill and showed clinical deterioration There was little
significant change in heart rate on venesection Blood pressure fell slightly
in 3 of the observations

Effect of intravenous digoxin (1 5 mg) Results are shown in Table III
and an example in Fig 2 There was no significant change in arterial
oxygen saturation Right auricular pressure fell in every case In 3 of
the 5 cases cardiac output decreased In Case 13, cardiac output showed
a conspicuous increase, and simultaneously the mean right ventricular
pressure increased from +21 5 cm to +53 cm This case was severely ill
and had shown only a small rise of blood pressure in an oxygen tent (Table
II) Case 17, whose initial blood pressure was also low, showed a similar
rise of output and blood pressure after digitalis There was no significant
change in heart rate

DISCUSSION

The results indicate that the responses of Group A are different from those in heart failure due to hypertensive, ischæmic and valvular heart disease   First, cardiac output is increased instead of decreased   This has been previously recorded briefly by McMichael and Sharpey-Schafer (8) and by Richards (10)   Secondly, the usual response to a fall of venous filling pressure from venesection or digitalis is a decrease in cardiac output instead of the usual increase observed in cases of low output heart failure with an initially high venous pressure (8, 5)   In severe anæmia the oxygen carrying power of the arterial blood is reduced while cardiac output is increased as a result of a raised venous filling pressure and moderate increase in heart rate (12)   Somewhat analogous conditions are found in Group A   available arterial oxygen is reduced, while a high cardiac output is maintained by an increased filling pressure and moderate increase in rate   In severe anæmia the usual response to a fall of venous pressure is a fall in cardiac output, and a rise of venous pressure also leads to a fall (13)   For obvious reasons, data on the effect of raising venous pressure still further are not available in cases of heart failure from emphysema, but the effects of lowering venous pressure resemble those in anæmia   It is suggested that the usual status of the two conditions on Starling's venous filling pressure-cardiac output curve is similar (5)   Rarely, the heart in severe cases of anæmia and emphysema lies on the overloaded or falling part of the curve

Group B, with low blood pressure, represent a terminal phase, and clinical experience of a number of other cases in this phase has shown that death will follow in a few hours if no treatment is given, and that only a few cases will respond favourably to treatment   Fig 3 shows that the low arterial pressure is largely determined by a low output which has presumably fallen from a previously higher level

The data reported in this paper are limited to cases with raised right auricular pressures ,   observations on earlier cases may help to show the relative importance of the parts played by a rise of pulmonary arterial pressure and a falling arterial oxygen saturation in the production of right heart failure in chronic bronchitis and emphysema   An important contribution to the problem of right ventricular hypertrophy in cor pulmonale has recently been made by Bloomfield and others (1) who have shown that in emphysema, before the development of heart failure, the pulmonary systolic pressure rises from a normal of 18 to 30 mm Hg to 34 to 57 mm Hg   No further increases in pulmonary arterial pressure were recorded in the presence of failure   Other forms of heart failure due to mitral stenosis, ischæmic heart disease and syphilitic aortic incompetence showed as high, or even higher, pulmonary arterial pressures (up to 103 mm Hg in one case of mitral stenosis), particularly when failure was present

It is of interest that consciousness and blood pressure can be maintained in these cases at levels of arterial oxygen saturation below 50 per cent,

since acute reduction of arterial oxygen saturation in normal subjects below 60 per cent results in unconsciousness and, if prolonged, in death   A slow process of tissue adaptation may be involved

Occasional enlargement of the left ventricle in heart failure from emphysema has been found at post mortem (3, 11)   Parkinson and Hoyle (9) considered such enlargement to be due to pre-existing hypertension   The finding of an increased cardiac output makes it possible that some cases of left ventricular enlargement result from a chronic increase in cardiac work The increase in work of the heart as a whole may be a contributing factor in the development of the terminal pulmonary œdema which is sometimes observed, and is a frequent occurrence in the similar condition of fatal anæmia

Practical considerations   The observations indicate that raised venous pressure and increased cardiac output are to be regarded as compensatory phenomena and that harm is likely to result from lowering venous pressure and output   In the past we have observed cases who showed rapid deterioration following venesection or digitalisation   Although many authors advise these therapeutic measures in heart failure from emphysema (6, 15), others such as Wollheim (16) were of the opinion that they were of no value   Venesection is often tempting because of the " congested " appearance of these patients, and the ease of withdrawing blood   Although the patient may appear less cyanotic at the end of venesection, this is probably due to constriction of the small vessels of the skin, since measurement of arterial oxygen saturation shows no change   Digitalis might, however, be used in those rarer severe cases when the response of the heart indicates that it is on the falling or overloaded part of Starling's curve   Unfortunately actual measurements of cardiac output are necessary to establish this Temporary lowering of venous pressure by venous tourniquets on the legs may give the required information   Digitalis might also be given for its blood pressure raising action in cases where low blood pressure is not sufficiently restored by giving oxygen

The measure of greatest immediate value is the administration of oxygen   The rise of blood pressure and slight fall of cardiac output indicates an increase in total peripheral resistance presumably due to arteriolar vasoconstriction   We have maintained cases in an oxygen tent for more than a month, and if there is some other factor such as an exacerbation of respiratory infection which can improve, then there is a chance that life may be prolonged   Another procedure that seems to be of value is reduction of thyroid activity with thiouracil (14)   In a few cases arterial oxygen saturation may rise to a considerably higher level, though remaining subnormal, the venous pressure may fall to normal and the patient improve sufficiently to leave hospital

SUMMARY

1 Studies of the circulation were made in 14 cases of emphysema and 3 other cases of chronic pulmonary disease with raised right auricular pressure The cases fall into two groups A with normal blood pressure B with systolic blood pressure below 90 mm Hg and a poor immediate prognosis

2 In group A cardiac output was increased, in group B it was usually decreased Mean right ventricular pressure was increased in the 5 group A cases in which it was measured Chronic increase in the work of the heart may explain occasional examples of left ventricular enlargement

3 Arterial oxygen saturations below 50 per cent were observed in some cases Administration of oxygen raised the arterial oxygen saturation, increased the blood pressure and usually caused a small fall of cardiac output

4 When right auricular pressure was lowered by venesection or intravenous digoxin, cardiac output usually fell In 3 severe cases cardiac output increased The blood pressure may rise after digoxin although cardiac output decreases

5 Group A cases differ from cases of low output heart failure (hypertensive, ischaemic and valvular heart disease) in that resting output is increased and the response to a fall of venous filling pressure is usually a decrease in cardiac output In these responses they are similar to cases of severe anaemia Therapeutic measures which lower venous pressure (venesection and digitalisation) are therefore usually harmful Prolonged sojourn in an oxygen tent, treatment of any respiratory infection, and depression of thyroid activity by thiouracil are the measures most likely to prolong life

APPENDIX

S R = sinus rhythm Voltage of P wave in Lead II is given in mv The liver was palpable in all cases All cases examined at post mortem (except Case 15) showed hypertrophy and dilatation of the right ventricle, and enlargement of the right auricle

Case 1 Male, aged 69 Winter bronchitis 20 years Dyspnoea 6 years Oedema 4 months Cyanosis, oedema and ascites E C G S R Right axis shift ST₂ depressed P₂0 1 mv Death 8 hours later Post mortem heart weight 470 g Left ventricle slight hypertrophy Lungs emphysema

Case 2 Male, aged 31 Asthma and bronchitis since infancy Oedema 1 year Cyanosis, oedema and ascites E C G S R , later paroxysmal ventricular tachycardia, right axis shift, low voltage P₂ 0 3 mv X ray pulmonary arteries enlarged Conus prominent Considerable enlargement of heart Blood R B C 5,200,000 per c mm Hb 93% No improvement after bed rest and mercurial diuretics Venous pressure normal after thiouracil Nine months later working, venous pressure normal, arterial oxygen saturation 84%

Case 3 Male, aged 42 Chronic bronchitis and asthma 10 years Cyanosed, irritable and forgetful 1 year Deep cyanosis, oedema and ascites Vital capacity 1,100 c c E C G S R Right axis shift ST₂ depressed P₂ 0 25 mv X ray pulmonary arteries enlarged Heart moderate enlargement Blood R B C 6,300,000 per c mm Hb 93%· Numerous fundal haemorrhages Papilloedema developing later C S F Pressure greater than 300 mm No

improvement from oxygen and thiouracil Death after 2 months Post mortem heart weight 440 g Left ventricle normal. Lungs emphysema, slight atheroma of pulmonary artery intimal thickening of small branches of pulmonary artery

Case 4 Male, aged 52 Persistent cough 30 years increasing dyspnœa 5 months Cyanosis, œdema and slight ascites Vital capacity 700 c c E C G S R right axis shift $ST_{2, 3}$ depressed. P_2 0 23 mv Blood R.B C 6,300 000 Hb 110% Improved with rest and thiouracil, left hospital. Died with acute respiratory infection Post mortem heart weight 550 g Left ventricle slight enlargement Lungs emphysema

Case 5 Female aged 54 Asthma and bronchitis for 23 years severe dyspnœa 6 months, cyanosis, œdema and ascites E C G S R Low voltage, right axis shift P_2 0 35 mv X ray pulmonary arteries enlarged Slight prominence of conus Moderate enlargement of heart Blood R B C 4,800,000 per c.mm Hb 87% Oxygen tent 3 weeks Improved after thiouracil Venous pressure normal Arterial saturation 87% Left hospital Death 6 months later Post mortem heart weight 450 g Left ventricle normal Emphysema

Case 6 Male aged 56 Dyspnœa many years Cyanosis and œdema E C G S R Right axis shift $ST_{2, 3}$ depressed P_2 0 35 mv Death 24 hours later Post mortem heart weight 415 g Left ventricle normal. Slight atheroma of coronary arteries Lungs emphysema and chronic bronchitis

Case 7 Male aged 61 Bronchitis and asthma 12 years Dyspnœa 6 years Oedema 1 year Cyanosis, œdema and ascites E C G S.R Right axis shift Low voltage P_2 0 1 mv X ray whole heart greatly enlarged Blood R B C 5 000,000 per c.mm. Hb 100% No improvement Death after 1 month Post mortem heart weight 470 g Slight hypertrophy of left ventricle Lungs emphysema

Case 8 Female aged 30 Asthma for 12 years Dyspnœa and œdema 1 year Cyanosis and œdema Vital capacity 600 c c E C G nodal rhythm Right axis shift $ST_{2, 3}$ depressed P_2 increased voltage X ray enlarged pulmonary arteries conus and right ventricle present a year previously Same findings with additional moderate enlargement Blood R.B C 10 500 000 per c.mm Hb 128% Death 1 month later Post mortem heart weight 500 g Slight hypertrophy of left ventricle Atheroma of pulmonary arteries Lungs emphysema

Case 9 Male, aged 56 Winter bronchitis many years Dyspnœa and œdema 2 months Cyanosis and œdema E C G S R later showed paroxysmal auricular flutter and fibrillation. Right axis shift $ST_{2, 3}$ depressed. P_2 0 1 mv X ray pulmonary arteries enlarged. Conus not prominent Moderate enlargement of heart Blood R B C 4 700 000 per c.mm. Hb 96% Appeared moribund soon after admission. Kept in oxygen tent for 1 month. Improved after thiouracil right auricular pressure falling to —7 cm and cardiac output to 5 4 litres per minute but arterial oxygen saturation remained about 65 per cent Venous pressure normal, and able to get about for 6 months Death in a few days from acute respiratory infection. No post mortem

Case 10 Male aged 30 Dyspnœa recurrent bronchitis and pneumonia since childhood. X ray bilateral congenital cystic disease of the lungs Right axis shift (E C G) and enlarged pulmonary arteries 1 year previously Cyanosed, minimal œdema Vital capacity 1 250 c c E C G S R Right axis shift $ST_{2, 3}$ depressed P_2 0 2 mv X ray pulmonary arteries enlarged conus prominent Moderate enlargement of heart Blood R.B C 5,200,000 Hb 83% Treated with thiouracil Alive 4 months later

Case 11 Male, aged 55 Chronic bronchitis for 15 years Oedema 3 weeks Cyanosis and œdema E C G S R Right axis shift $ST_{2, 3}$ depressed. P_2 0 15 mv X ray pulmonary arteries enlarged. Moderate enlargement of heart Blood R.B C 4,000 000 per c mm Hb 95% Improved in oxygen tent Alive 2 months later

Case 12 Male, aged 48 Asthma and bronchitis for years Cyanosed, very ill, œdema E C G S R Low voltage Right axis shift P_2 0 15 mv Death 6 hours later Post mortem heart weight 400 g Left ventricle normal Slight atheroma of pulmonary artery Lungs emphysema œdema and purulent bronchitis

Case 13 Male aged 44 Dyspnœa and œdema 4 weeks Gross kyphosis Cyanosis and œdema E C G S.R Low voltage Right axis shift ST_2 depressed P_2 0 1 mv X ray gross kyphosis and scoliosis Some improvement in oxygen tent and after digitalis, which was not maintained Death 12 hours later No post mortem

Case 14 Male aged 60 Dyspnœa and bronchitis for years Cyanosis and œdema E C G sinus rhythm Right axis shift ST_2 depressed P_2 0 2 mv Death 12 hours later Post mortem heart weight 465 g Left ventricle normal Lungs emphysema and patchy bronchiectasis

Case 15   Male, aged 51   Welsh miner   Silicosis, cough, hæmoptysis and dyspnœa for years   Cyanosis and œdema   E C G   S R   Low voltage, right axis shift P_2 0 15 m\   X ray   silicosis of lungs, pulmonary arteries enlarged, conus prominent, moderate enlargement of heart   Death 12 hours later   No post mortem

Case 16   Female, aged 55   Carcinoma of breast   Dyspnœa and œdema 2 weeks   Cyanosis and œdema   Post mortem   heart weight 210 g   Right auricle dilated   Other chambers atrophied   Lungs   both lungs shrunken, widespread infiltration of lung lymphatics by carcinoma

Case 17   Male, aged 66   Welsh miner   Dyspnœa and bronchitis for years   One year previously X ray showed slight enlargement of pulmonary arteries and heart   Very ill   Cyanosis and œdema   Blood   Hb 97%   X-ray   pulmonary arteries and conus enlarged   Moderate enlargement of heart   Slight improvement after digitalis   Death 10 days later   Post mortem heart weight 381 g   Left ventricle normal   Lungs   emphysema, anthracosis and some fibrosis

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RENAL FUNCTION IN ADDISON'S DISEASE *

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IT is well known that retention of urea and other nitrogenous substances may occur in Addison's disease, and that this may be corrected by adequate treatment with sodium salts and the hormones of the suprarenal cortex This azotæmia is not explicable on the basis of structural changes in the kidney, and it seemed that the use of the inulin and diodone clearance methods devised by Smith and his colleagues (17) might reveal its mechanism, and at the same time provide some information about the action of the cortical hormones on the kidney   The work was begun in ignorance of the paper of Talbott, Pecora, Melville and Consolazio (18), who used these methods to investigate the same problem   The present work in the main confirms, and somewhat amplifies, their results, which were briefly as follows In 10 patients with Addison's disease, and 6 patients described as having " chronic adrenal insufficiency associated with pan-hypopituitarism," inulin clearance and diodone clearance were both depressed below normal, the former more than the latter, so that the filtration fraction tended to be low Maximum tubular reabsorption of glucose was severely depressed, and maximum tubular excretion of diodone to a lesser extent Administration of desoxycorticosterone acetate corrected partially, but temporarily, these deficiencies , administration of adrenal cortical extract had no demonstrable effect   Sodium   clearances   failed   to   demonstrate   inadequate   tubular reabsorption of sodium, and showed no consistent alteration after treatment , potassium clearances, however, showed an increase after treatment in 5 out of 6 patients studied

Methods and material

Full details of procedure for determining inulin and diodone clearances and maximum tubular excretion of diodone (Tm_D) have been given by Goldring and Chasis (5)   The procedure used in the present work was

similar, apart from the following points   the bladder was washed out with two lots of 50 ml of distilled water when obtaining urine specimens, the patient received no extra water by mouth before the clearance observations, and the plasma proteins were removed by the ordinary tungstic acid procedure, as this was found to give the smallest loss of diodone, although even in this procedure a loss of 3% of the plasma diodone had to be allowed for

The following chemical methods were used   Inulin   Hubbard and Loomis (7), Diodone   Alpert (1), Sodium   Morton (11), Blood urea Conway and O'Malley (3)

Hæmatocrit readings were taken after centrifuging for 30 minutes at 3,000 r p m , no correction was made for plasma trapped in the cell column Plasma volume was determined in one case with T-1824, the dye concentration being determined directly in serum with a photo-electric absorptiometer Blood samples for this were drawn at the same time as those required for the clearance experiment, and a dye disappearance curve constructed in the usual way   Fluid shifts due to the saline infusion used for maintaining inulin and diodone plasma levels were allowed for as described by Noble and Gregersen (12), the serum protein being measured by Van Slyke's (13) copper sulphate method

It is convenient to note at this point a troublesome feature of Alpert's diodone method which requires special technique   Filtrates of plasma or serum drawn before the injection of any diodone give a " blank " titration value amounting to some 0 3 mg diodone-iodine per 100 ml   This effect has also been observed by Shannon and co-workers (4)   In itself this would be no great drawback, were it not that the blank titration value is a function of the time that has elapsed after the addition of the potassium iodide To overcome this difficulty, it was found necessary to construct a curve for each plasma, relating the titration value of the blank to the time elapsed since the addition of iodide   Samples containing diodone were then titrated, the time from the addition of iodide to the endpoint being noted   The blank value corresponding to this time was found from the curve and subtracted from the titration value   Only in this way was it possible to obtain consistent results with plasma   The effect was not observed with urine *

Four patients were investigated , their clinical details are appended In each case, the inulin clearance, diodone clearance and TmD were first determined   treatment was then begun with sodium chloride by mouth and desoxycorticosterone acetate (DOCA) by intramuscular injection, and the tests were repeated after satisfactory clinical improvement   An average dose of 5 g sodium per day was given in addition to that present in the diet , the initial dose of DOCA in all cases was 5 mg per day, this being subsequently adjusted to the individual's requirements   The only case

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* The writer is grateful to Mr N C Hughes Jones who kindly investigated this matter and evolved the final procedure

requiring less than this amount was H B , who developed œdema two weeks after the commencement of treatment   thereafter she received 5 mg of DOCA every other day

No evidence of intrinsic renal disease was found in any of the cases, and none presented signs of Addisonian crisis

Results

In Table I, summarising the results, all clearance values and Tm's are corrected to the standard surface area of 1 73 sq.m , and the values obtained from normal subjects by Goldring and Chasis (5) are given for comparison

TABLE I.

Pt	Sex	Age	Date	BP mm Hg	Serum sodium mg per 100 ml	Blood urea mg per 100 ml	Hæm ato crit %	C_{IN} ml / min	C_D mL./ min	Tmp* mg.I/ min.	Filtn. fract %
E F	F	34	1 3 46	95/60	—	35	35	67	456	26 0	14 7
			8 4 46	110/70	330	24	35	70	370	28 5	18 9
R B	F	33	14.3 46	88/55	297	35	39	64	347	39 1	18 4
			2 5 46	94/54	344	14	36	95	411	—	23 1
H B	F	38	2 4 46	100/58	320	29	33	68	422	32 9	16 1
			22 5 46	122/74	313	20	28 5	64	374	-39 4	17 1
E D	M	43	22 7 46	82/60	290	59	42	73	530	37 2	13 8
			19 8 46	124/68	332	21	35	147	556	42 7	26 5
					Normal Values (11)		M	131	697	51 8	19 ± 2
							F	117	594	42 6	20 ± 3

* Maximum tubular excretion of diodone

In Table II is shown the percentage change after treatment in the various functions measured

The following points may be noted   Firstly, the inulin clearance was subnormal in all cases before treatment   The effect of treatment was variable   in R B and E D there was a rise, but in the other 2 cases there was no significant change

The diodone clearance was also subnormal in all cases before treatment, and again the result of treatment was variable

TABLE II

Patient	Δ C$_{IN}$ %	Δ C$_D$ %	Δ Tm$_D$ %	Δ FF %
E F	+ 6	− 10	+ 10	+ 28
R B	+ 48	+ 18	—	+ 26
H B	− 3	− 11	+ 20	+ 7
E D	+ 100	+ 5	+ 15	+ 92

The filtration fraction (calculated as $\frac{\text{Inulin clearance}}{\text{Diodone clearance}}$) was low or low normal before treatment (as observed by Talbott and co-workers), and in all cases the alterations in inulin clearance and diodone clearance following treatment were such as to raise the filtration fraction, even, in E D, to the probably abnormal figure of 26%

TABLE III.

Patient	Date	Sodium excretion %
E B	14 3 46	3 6
	2 5 46	2 1
H B	2 4 46	1 7
	22 5 46	3 4
E D	22 7 46	1 8
	19 8 46	1 3

Tm$_D$ was low in 3 cases before treatment   in the 3 cases in which it was measured on a second occasion it had risen significantly, in H B almost to normal levels

Sodium reabsorption   In 3 cases the tubular reabsorption of sodium was calculated as follows   The sodium entering the tubules can be found by multiplying the inulin clearance by the serum sodium level, the sodium leaving the tubules is obtained by multiplying the urine flow by the urinary sodium value   Table III shows the percentage excreted of the total sodium entering the tubules   Current theories of Addison's disease would lead one to expect diminished excretion after treatment, but in fact the differences observed were not striking and in one case the excretion increased after treatment   It is, however, to be pointed out that extra salt was given by mouth during treatment, and that the comparison made was not simply that of the behaviour of a given kidney with and without DOCA   Talbott and co-workers were also unable to draw any definite conclusion from their

figures for sodium clearance , in their series the patients were receiving added sodium chloride by mouth at the time of the first clearance test, and this was presumably continued after treatment with DOCA was begun, though this is not explicitly stated   The amount of salt given to these patients is also not stated , it may be that a sufficiently large daily dose would so increase the urinary sodium excretion as to mask any effects of DOCA

Discussion

The inulin and diodone clearances   The main problem with which this work is concerned, namely the mechanism of the azotæmia in Addison's disease, appears to find at least a partial answer in the extremely low inulin and diodone clearances found in untreated cases   The inulin clearances ranged from 55% to 58%, and the diodone clearances from 58% to 77%, of normal   From the evidence of Smith and others (15) it may be accepted that these results imply proportionately low values for the glomerular filtration rate and the effective renal plasma flow in untreated Addison's disease

The maximum tubular excretion of diodone   Smith (17) originated the conception of Tm_D when working on essential hypertension, in which a gradual reduction of the mass of functioning tubular tissue takes place with the passage of time   In Addison's disease no constant structural alterations in the kidney are known, and the lowered Tm_D must be evidence of a purely functional impairment of the renal tubules   This is at any rate partly corrected by treatment   It is of interest that Welsh and co-workers (20) described increase in Tm_D of the dog's kidney following injection of testosterone, a substance closely related structurally to desoxycorticosterone   On the other hand, Klopp, Young and Taylor (9) gave large doses of testosterone to normal men and one eunuchoid man, and found no change in maximum tubular excretion of p-amino-hippuric acid

The effects of treatment   It will be seen from Table I that only in one case (E D) did the clearance figure obtained after treatment approach normal , in the remaining cases only a partial restoration of function was achieved   Several explanations of this are possible   It may be that the lesion is only slowly reversible, and that insufficient time was allowed for recovery   Other examples of slowly reversible extrarenal azotæmia are known, for instance the varieties found in shock (10) and in alkalosis (8)   It should be noted, however, that the case showing the most complete restoration to normal had, of the whole series, the shortest interval between tests

Another possibility is that the substitution therapy given was inadequate, either because the substance used did not completely replace the secretion of the suprarenal cortex, or because the dosage was too low   It is well known that the suprarenal cortex produces a number of steroid hormones,

and it is probable that the various functions of the gland are accomplished by different hormones, or at any rate different groups of hormones Thus it is known that desoxycorticosterone, the compound used in treating these patients, has no demonstrable effect on carbohydrate metabolism, although it has a marked effect on the absorption and excretion of sodium and potassium (19) It might be argued, therefore, that some cortical hormone was missing, whose presence would have brought about complete restoration of normal renal function This possibility appears unlikely in the light of the observations of Talbott and co-workers They gave 2 of their patients 20 c c of adrenal cortical extract (Wilson's) daily by intramuscular injection for 5 days, and found no significant change in inulin clearance or diodone cleaiance These patients had both had implantations of DOCA 12 months previously (494 and 515 mg) and were in a satisfactory clinical state The authors concluded that " adrenal cortical extract in the doses given was unable to restore impaired renal function in patients with Addison's disease beyond that achieved by desoxycorticosterone acetate " With legard to the dosage of DOCA employed in the present series, it is perhaps worth noting that H B , whose inulin and diodone clearances both fell slightly with treatment and whose filtration fraction showed the smallest rise in the series, also had the smallest dosage of DOCA

The possibility must also be considered that in Addison's disease, some permanent structural kidney damage develops, which no replacement of missing hormones can repair Barker (2) has reported atrophic changes in the tubules in 10 out of 31 cases of Addison's disease which came to autopsy , these changes consisted of flattening of the epithelium, intertubular œdema, and occasionally the presence of fat droplets in the tubular cells On the other hand, Guttmann (6) who compiled a series of 566 autopsied cases of Addison's disease from previous reports, listed only 33 cases in which lesions of the kidney, other than tuberculous, were found It must be remembered that autopsy reports of cases of Addison's disease prior to 1939, when synthetic DOCA became available, are almost certainly reports of patients dying in Addisonian crisis

It is possible that the tubular lesions found by Barker were due to the severe circulatory collapse occurring in this condition, and not to any adverse effect on the kidney of the chronic suprarenal insufficiency exhibited by the patients in this series

The rise of filtration fraction with treatment Smith (16) has shown that wide departures from the normal filtration fraction can be induced by pyrogenic agents and by adrenaline Pyrogens cause a rise in diodone clearance and adrenaline a fall, but in both cases the inulin clearance remains unchanged The simplest explanation of these effects is that there is an alteration in the tone of the efferent glomerular arterioles, adrenaline constricting and pyrogens dilating them It seems likely that the rise in filtration fraction induced by treatment in all 4 cases may also be due to

increased tone of the efferent arterioles, and if this is so, we must suppose that in the untreated cases there was a relaxation of this tone, which was more or less corrected by treatment   Talbott and co-workers reached a similar conclusion   But examination of the results in Table I will show that this cannot be the only readjustment taking place in the kidney   A simple constriction of efferent arterioles would be expected to diminish the diodone clearance and leave the inulin clearance unchanged, and this is in fact what occurred in cases E F and H B   But the other two cases show a slight rise in diodone clearance, in addition to a conspicuously increased inulin clearance, and for this to happen in the face of constriction of the efferent arterioles there must have been a rise in perfusion pressure of the glomeruli   The most likely way in which this could happen is through a rise in systemic arterial pressure, and this is seen to have been marked in the case of E D   But the effect can occur with only insignificant rise in blood pressure, as the case of R B shows, and here we must conclude that some dilatation of the afferent glomerular arterioles occurred

We may now enquire what is the cause of the change in efferent arteriolar tone   The effect is probably not a simple response to a rise in blood pressure   R B showed a conspicuous alteration in filtration fraction, but the blood pressure was little changed ,   H B , who showed the smallest change in filtration fraction, had a well marked rise in blood pressure Moreover, in several of the untreated cases reported by Talbott and co-workers the filtration fraction was very low at a time when the blood pressure was normal

Changes in plasma volume are also unlikely to be the explanation The plasma volume was measured only in the last case, in which it rose from 2410 ml before treatment to 3070 ml after treatment , but the percentage change can be calculated in the other 3 cases from the haematocrit readings which were taken on the occasion of each test   The change in plasma volume in the last case as measured directly with the dye T-1824 agrees almost exactly with the percentage change calculated from the haematocrit values, so the latter procedure would seem to be reliable provided that the total cell volume remains constant   Unfortunately, the three cases in which indirect measurement alone was possible were females, but there is no record of the menstrual loss being excessive in any of them, and no other source of blood loss was found   If this indirect method is accepted, it will be seen that the second largest increase in plasma volume is associated with the smallest change in filtration fraction (H B), and that a case with a large change in filtration fraction had apparently no alteration in plasma volume (E F)

The serum sodium level appears to have some correlation with the filtration fraction, but the series is too small for any conclusion to be drawn The final possibility is that the rise in filtration fraction may be a direct effect of DOCA on the kidney, but this theory cannot be advanced with any

certainty since treatment included sodium chloride by mouth as well as injection of DOCA However, Talbott and co-workers found low filtration fractions in patients treated with salt before any DOCA had been given Their results do not permit of any definite conclusions as to the effect of DOCA on the filtration fraction

SUMMARY

1 Renal function was investigated in 4 cases of Addison's disease not in crisis, using the inulin and diodone clearance technique

2 The inulin clearance, diodone clearance and diodone Tm were depressed in untreated cases , the effect of treatment on the first two functions was variable, but in all cases was such that the value of filtration fraction ($\frac{\text{inulin clearance}}{\text{diodone clearance}}$), previously low, was increased The diodone Tm was increased after treatment

3 The possible significance of these findings is discussed

Clinical notes

E F , female, aged 34 Eighteen months before admission she began to have frequent epistaxis, and six months later she noticed severe weakness, which became progressively worse , she also became short of breath on exertion Six months before admission she noticed that her skin was becoming darker than usual, the face being the part earliest affected Her legs and feet have always been rather dark, but during the last few months before admission her whole body became involved She had no diarrhoea or vomiting, but experienced occasional nausea

On admission, her skin was fairly deeply pigmented with jet-black freckles on the face and arms No pigmentation of the mucous membranes could be discovered Blood pressure 128/80 The chest was normal clinically, except for some fine crepitations at both bases

Chest X ray showed small bilateral pleural effusions these were not detectable clinically On 16 3 46 she complained of pain in the right lower chest and had a temperature of 101° , it was found that the effusion in the right pleural cavity had increased considerably The fluid when aspirated was found to be straw coloured, containing a few cells, 60% of which were neutrophil polymorphonuclear leucocytes Culture for tubercle bacilli was negative The temperature subsided rapidly, being normal on 21 3 46 , the effusion also disappeared quickly and a chest X ray on 1 4 46 showed no fluid in either pleura

On 13 4 46 200 mg DOCA was implanted into her abdominal wall, and the injections were discontinued She was discharged on 28 4 46, feeling well, with a blood pressure of 110/60

R B , female, aged 33 First admitted 18 months previously, complaining of weakness, pigmentation of skin, amenorrhoea, and loss of weight She also had occasional vomiting and some attacks of giddiness

On examination there was general pigmentation, marked in the axillae and the perinoum, with very striking involvement of the buccal mucosa and the lips Blood pressure at this time was 70/50 mm Hg Blood chemistry Serum sodium 318 mg /100 ml fasting blood sugar 90 mg /100 ml

Chest X-ray old fibrotic and calcified lesion in left upper zone, and evidence of old pleurisy at right base

She was treated with sodium chloride by mouth and DOCA by intramuscular injection , finally 200 mg DOCA was implanted into the right rectus sheath and she was discharged 10 days later, with B P 105/75

She remained well until 3 weeks before the present admission, when she noticed return of her asthenia On admission the B P was found to be 74/50 The pigmentation had not changed since her previous admission Serum sodium 294 mg /100 ml , serum potassium 19 6 mg /100 ml Fasting blood sugar 80 mg /100 ml

H B , female, aged 38   In September 1945, this patient was admitted with an abscess in the left breast   this was aspirated and tubercle bacilli were seen in the pus   She was discharged but 3 months later the abscess burst spontaneously and continued to discharge pus for another 3 months

While she was ill with the breast abscess she lost 19 lbs   from December, 1945, until her admission on 29th March, 1946, her weight remained about constant

In January 1946, she noticed that her skin was becoming dark, and in February noticed ready fatigue even on standing   also slight dyspnœa on climbing stairs   About this time she began to vomit occasionally, usually at mid day

On examination, there was pigmentation on the backs of the hands, over the knuckles, on the palmar surfaces of the fingers in the flexures   on the shoulders at the sites of pressure from shoulder straps   and in the mouth, inside the cheeks and on the soft palate   The blood pressure was labile , on one occasion a reading of 100/56 was obtained, and on another 115/20 Other observers occasionally obtained values higher even than this second value   The chest was normal clinically

Blood chemistry

Venous plasma CO_2 content	60 vols %
Plasma chloride	373 mg /100 mL.
Plasma sodium	320 mg /100 ml
Blood urea	29 mg /100 ml

Sedimentation rate 20 mm in 1 hour (Wintrobe)

E D , male, aged 43   Eight months before admission he began to notice nausea after meals, but no vomiting apart from that which he induced to relieve the nausea   For 2 weeks before admission he noticed severe asthenia   Bronzing of the skin had been apparent for some 3 months   during the same period he lost 6 lbs in weight

Past history   Eight years previously spent 3 months in a sanatorium for right pleural effusion and infiltration of right lung

On admission he showed general pigmentation, on the face, backs of hands and palmar creases   also in scars on the trunk caused by a flying bomb incident 2 years previously   Very slight pigmentation of gums   Blood pressure 90/60   Blood chemistry on admission

Venous plasma CO_2 content	51 vols %
Venous plasma chloride	332 mg /100 ml
Serum sodium	294 mg /100 ml
Serum potassium	21 7 mg /100 ml
Blood urea	53 mg /100 ml

Chest X ray showed old infiltration at right apex and more recent infiltration at left apex Sputum   no tubercle bacilli found at two examinations

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RENAL FAILURE FOLLOWING ABDOMINAL CATASTROPHE

AND ALKALOSIS

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COMPARISON between the results obtained by surgery in the recent war and in the war of 1914-18 reveals a conspicuous advance in every field save one—that of abdominal injuries   In spite of early treatment by forward surgical units, chemotherapy with penicillin and sulphonamides, and the many advances in surgical and anæsthetic technique, a penetrating wound of the abdomen still carries a mortality of some 30-40%   Edwards (11) reported from the Italian theatre 388 abdominal cases without other important injuries, with a mortality of 39 7%   Porritt (24), in a summary of abdominal cases in 21 Army Group for the period June to November, 1944, reported 2,479 cases of penetrating wounds, with a mortality of 30 7%   Such figures may well be due, in part at any rate, to the difficulty, peculiar to the abdomen, of controlling an infection which has once gained a foothold there   But a growing body of evidence suggests that in many of the fatal cases renal failure is a contributory, and possibly in some a dominant, factor   Edwards, in the series mentioned above (11), listed the causes of death in groups dying within varying periods after operation   Of 50 cases who died 2 to 10 days after operation, 13 succumbed to renal failure , this was nearly twice as common as any other single cause

Wounds of the abdomen are uncommon except in wartime   In times of peace, a close parallel is presented by the common surgical emergencies, such as perforation of a peptic ulcer, strangulation of the intestine, and in fact any condition likely to cause peritonitis, paralytic ileus, gross dehydration or a combination of these   Accordingly, in order to obtain further insight into the mechanism of renal failure, 4 cases of this nature have been investigated with the inulin and diodone clearance technique of Homer Smith (27)   A case of alkalosis due to excessive intake of alkali has also

been studied and is reported here, for, while alkalosis is well known to occur in the abdominal conditions already mentioned, its mechanism is likewise obscure

A full account of the inulin and diodone clearance methods and of the experimental work on which they are based may be found in the papers of Homer Smith and his colleagues (12, 27, 28) Briefly the rate at which the kidney clears the blood of inulin (C_{IN}) may be taken to measure the rate of glomular filtration , its value in normal adults is 100 to 130 ml per min   Diodone is excreted largely by the tubules , its removal from the plasma perfusing the normal kidney is so nearly complete that its clearance at plasma levels below 10 mg per 100 ml (C_D) may be used as a measure of the blood perfusing the secretory tissue of the kidney, or as Smith (27) puts it the " effective renal plasma flow ", in normal adults this value is 600-800 ml per min   The ratio $\dfrac{\text{inulin clearance}}{\text{diodone clearance}}$ is termed by Smith the filtration fraction (F F) since it represents the proportion of plasma water perfusing the excretory tissue which is filtered off in the glomeruli , a normal value is 17 to 23%   At plasma levels above 40 mg per 100 ml the tubular excretion of diodone reaches a maximum value which is not increased by further rise of plasma diodone   This maximum tubular excretion of diodone (Tm_D) provides an index of the excretory mass of the functioning tubular tissue   Its value in normal adults is 40 to 50 mg diodone iodine per minute

Trueta and his colleagues (29) have recently provided evidence that in the rabbit various stimuli, including the application of a tourniquet to a limb, and prolonged faradism of the sciatic nerve or renal pedicle, cause blood to be diverted from the cortex through the medulla of the kidney, probably through an arteriovenous shunt   It is difficult to say how closely these findings can be applied to man , the rabbit's kidney is known to behave quite differently from that of the dog, the rat and man during a water diuresis (Dickel and Heller (10)), and it may be that this shunt mechanism is peculiar to the rabbit   It appears that hæmorrhagic shock in the dog is not a stimulus adequate to produce this response, at any rate until the renal blood flow reaches very low levels indeed, for Phillips and his colleagues (23) have found that the extraction ratio of p-amino-hippuric acid is maintained constant at 0 87 until the blood flow falls to about 3% of its normal value Selkurt (25) has reported similar results   Nevertheless it is clear that if the shunt mechanism described by Trueta comes into play, the diodone clearance becomes meaningless as a measure of renal blood flow   Any inaccuracy may still be avoided by retaining the original conception proposed by Smith, namely " *effective* renal plasma flow," for the diodone clearance should still measure the rate of plasma flow through *secretory* tissue

METHODS

The general technique employed was that described by Goldring and Chasis (12), with the following exceptions no additional water was given by mouth before the tests, and the bladder was washed out with two 50 ml samples of distilled water at each urine collection Owing to the grave conditions of the patients, one or at most two clearance periods, 15 to 20 minutes in duration, were usually all that could be done In case 1 tubular function was assessed by measuring the maximum tubular excretion of diodone (" Tm_D ")

Plasma volume was estimated with the dye T-1824, serum levels of the dye being determined directly with a photo-electric absorptiometer No difficulty was encountered from hæmolysis or lipæmia Blood volume was calculated from plasma volume and packed cell volume, no allowance was made for plasma trapped in the cell column The hæmatocrit tubes were centrifuged at 3000 r p m for 30 minutes

The following chemical methods were used Blood urea Conway and O'Malley (7) and later Peters and Van Slyke (22) Hæmoglobin Clegg and King (5) Plasma chloride Sendroy (26) Plasma CO_2 content Conway (6) Serum sodium Morton (20) Urine urea hypobromite method (Harrison (13)) Urine chloride Volhard method (Peters and Van Slyke (22)) Inulin (plasma and urine) Hubbard and Loomis (14) Diodone (plasma and urine) Alpert (3)

Blood for plasma chloride and CO_2 content was drawn with minimum stasis under oil and centrifuged under paraffin, with dry heparin as an anticoagulant The plasma proteins were precipitated with tungstic acid This caused an average loss of plasma diodone of 3%, which was allowed for in the calculations

ALKALOSIS

Case report

Case 1 J G, male, aged 57, was first seen in the outpatient department in June, 1944, when he gave a typical history of duodenal ulceration during the past 12 years The arterial pressure at this time was 160/95 The diagnosis of duodenal ulcer was confirmed by barium meal, and treatment was begun with a " gastric " diet and an alkaline powder* 1 drachm T D S This treatment he continued regularly and until March, 1946, he was relatively free of symptoms During the summer however he began to vomit two or three times a week, and on the night of 7 10 46 he began to vomit black material resembling coffee dregs Altogether he thinks he brought up about a gallon during the night

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* Bismuth carbonate 1 part, sodium bicarbonate 3 parts, calcium carbonate 3 parts, magnesium carbonate 3 parts
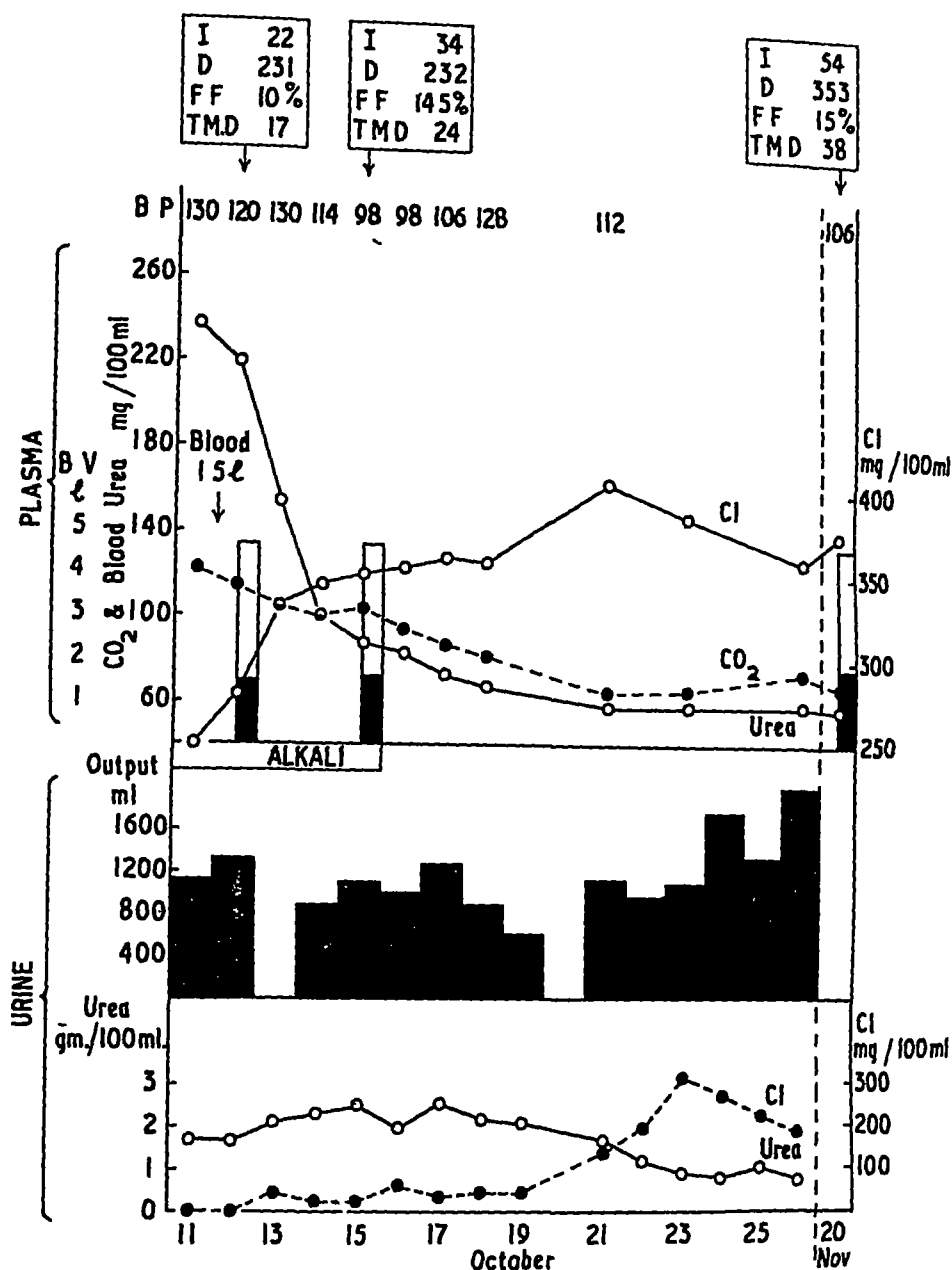
Fig 1   Summarise the biochemical findings in Case 1   In this and subsequent figures blood urea, plasma chloride and urine chloride are expressed in mg per 100 ml, plasma CO_2 as vols per 100 c c , urine urea as g per 100 ml   The vertical columns in the plasma section represent blood volumes, the black area representing cell volume, the white plasma volume The black rectangles in the urine section represent urinary output in ml per 24 hours Where no urine volume is indicated, the 24 hour specimen was wholly or partly lost through incontinence   The numerals in closed rectangles at the top of the figures represent the results of clearance determinations   I = inulin clearance, D = diodone clearance (both in ml/min), FF = filtration fraction and TmD = maximal tubular excretion of diodone in mg Iodine per min   Systolic blood pressure (S.B P) is in mm Hg

On admission the following day he was perfectly rational, with a blood pressure of 116/66   The urine was not examined   He was not vomiting nor did he vomit subsequently

In the evening of 8 10 46 he first began to show signs of mental disturbance , he could answer questions but frequently made irrelevant remarks   On the following day he was much the same, but later in the day became completely disorientated   The next day (10 10 46) he began to have visual hallucinations , he frequently tried to get out of bed but was easily controlled   However, that night he tried to strangle the night nurse

On 11 10 46 he was still completely disorientated and was having continuous visual hallucinations   There were signs of gross dehydration , the skin was inelastic and the tongue dry and very furred   The eyeballs were not unduly soft, and the arterial pressure was 153/90   Trousseau's and Chvostek's signs were negative, and there was no conjunctivitis, but blood examination showed gross alkalosis with considerable nitrogen retention, and the urine, which was pale, dilute and profuse in amount, contained only the faintest traces of chlorides   (*See* Fig 1)

During the night his condition deteriorated , Cheyne Stokes respiration developed and his arterial pressure began to fall, reaching 85/50 at 12 40 a m , 12 10 46   At 1 30 a m transfusion of stored blood was begun, 3 pints being given in all, followed by a slow infusion of normal saline, which was started at 9 15 a m   When clearances were done at 10 a m that morning he was perfectly quiet and rational, but occasionally incontinent of urine   His skin hydration was almost normal and his B P 120/60

From this point onwards his progress was satisfactory   The saline infusion was discontinued at 6 p m on 13 10 46 after a total of 3 litres had been given   Alkali by mouth was continued until 6 p m 15 10 46 after a second clearance had been done, and then stopped   Inulin and diodone clearances, diodone Tm and plasma volume were measured on 12 10 46, 15 10 46, and 20 11 46

Fig 1 shows several important points   (1) Following the infusion of blood and saline the blood volume was normal and remained so   (2) The clearance figures show a severe renal impairment, from which recovery was slow and, at the time of the last test, incomplete   (3) The urine secreted at the height of dehydration, the day before transfusion, was profuse and dilute (sp gr 1005   urea 1 7%)   (4) The urine chloride remained extremely low until 21 10 46, a full week after the plasma chloride returned to normal , at about the same time the plasma chloride rose to supernormal levels, and then, after a phase of increased urinary chloride excretion, returned to normal

Discussion

Two varieties of alkalosis are commonly encountered, the one due to loss of hydrogen and chloride ions through vomiting, and the other due to ingestion of excessive quantities of alkali Azotæmia is a frequent complication of both

Loss of plasma chloride can be made good to some extent by fixation of CO_2 as bicarbonate, but persistent vomiting, as for example in pyloric stenosis, ultimately leads to loss of plasma base as well as chloride, and this can only be restored by replacement from without Loss of base leads to loss of water, and therefore the alkalosis of persistent vomiting is regularly accompanied by dehydration From the little that is known of the effect of dehydration on the dynamics of the circulatory system and particularly on the renal circulation, and from cases 3 and 4 of the present series, it may be supposed that dehydration plays an important, perhaps a major, part in the causation of azotæmia in this variety of alkalosis A typical example, in which the renal function was investigated by inulin and urea clearances, has been reported by McCance and Widdowson (18)

In alkalosis due to the ingestion of alkali the incidence of dehydration is less certain, though in published reports of cases it is occasionally specifically mentioned In Case 1 dehydration was conspicuous at the time when alkalosis was first recognised The remaining cases here reported suggest strongly that dehydration may be an important factor in precipitating renal failure, and, although at the time of the first clearance determination in Case 1, the blood volume and tissue hydration had both been restored, the possibility cannot be excluded that the renal lesion was due to a pre-existing dehydration whose effects were only slowly reversible Nevertheless one feature of the renal lesion in this case is quite incompatible with dehydration being its sole or chief cause, namely polyuria Polyuria has been frequently, though by no means always, mentioned in published reports of alkali alkalosis, Cooke (8) states that "a diuresis of 2,000-3,000 c c is not uncommon," and Cope (9) gives the mean daily output of his third case when on alkali as 76 ounces, over 2 litres, urine flows were not, however, unduly large in Nicol's (21) cases Taken by itself the urine flow in the present case is not particularly striking but when it is realised that it was produced at first in the face of gross dehydration, the figures will be seen to be highly unusual (Compare the scanty initial output of Case 5) It would seem very probable that the dehydration, observed when alkalosis was first recognised in this case, was largely due to the polyuria, the patient was mentally abnormal for 3 days before the observations began and his fluid intake may not have kept pace with his output of urine, during this time he did not vomit

The renal disturbance in this case is remarkable for one other feature— the slowness of recovery This also appears to be a common finding in

alkalosis, but whether it is peculiar to that condition is not certain
Cooke (8) states that improvement in renal function (as measured by the
urea concentration test) often continues over several months after relief
of the alkalosis   Kirsner and Palmer (15), using the urea clearance test,
found that recovery usually took 7-14 days, but in 3 out of 25 cases it took
1, 3 and 6 months respectively, and in 2 cases the renal impairment was
persistent

The severe depression of the maximum tubular excretion of diodone
in this patient (to 17 as compared with the normal 40-50 mg iodine per min)
shows that tubular function was much impaired   Kirsner, Palmer and
Humphreys (16) found in 3 fatal cases of alkalosis degenerative changes
in the tubules often with flattening of the epithelium and widening of the
lumen, and very similar changes were present in Nicol's (21) case 4   Granular
casts are a common finding in cases of alkalosis and were in fact found in
the present instance   A specific toxic effect of alkali on the tubular epithelium
may thus be postulated, but would not explain the very low inulin clearance
The only explanation which fits the overall picture of renal function revealed
in this case is that of renal ischæmia resulting either from widespread renal
vasoconstriction or from the cortico-medullary shunt described by Trueta
and his colleagues (29)   If the presence of toxic amounts of alkali in the
blood were to act as one more stimulus for the partial exclusion of the kidney
from the circulation, the kidney lesion in alkalosis would indeed be due to
renal anoxia as suggested by Maegraith, Havard and Parsons (19) and
would fall into line with the condition found in dehydration and in so-called
shock

Abdominal catastrophe

Case reports and results

Case 2   B C , male, aged 58   At about 11 p m   on 26 12 45 an
embolus became lodged in the bifurcation of this patient's aorta
Embolectomy was performed at 3 a m   on 27 12 45   When seen at about
3 p m   that day, the patient's legs were cold and stiff and he could not move
them , they were stained with purplish patches and anæsthetic below the
knee   The blood pressure was 150-130/90 and auricular fibrillation was
present   The blood urea was 69 mg /100 ml

On 28 12 45 his condition was much the same but he was complaining
of severe abdominal pain and had begun to vomit   The blood pressure
was 150/100   He died at 11 p m

Post-mortem examination showed ascending thrombosis of the aorta,
with extension of the clot into the mesenteric arteries and infarction of the
intestine   The renal arteries were not involved and the kidneys were
normal macroscopically   No kidney sections were kept for microscopy

Clearance tests were carried out 8 hours before the patient died the results were as follows ᵇ

Inulin clearance 23 ml /min }
Diodone 60 ml /min } Filtration fraction 38%

The urine output on the day of the test was 390 ml in 24 hours

Comment This case presented two gross abnormalities, namely infarction of the lower limbs and infarction of the gut, and it is not possible to say which was responsible for the gross reductions in glomerular filtration rate and effective renal plasma flow It is probable from the nature of these lesions that the plasma volume was reduced It is noteworthy that the arterial pressure was not below normal (though a previous hypertension cannot be excluded) and the reduction in effective renal plasma flow was therefore probably due to renal vaso-constriction

Case 3 G E , male, aged 33 This man was admitted on 13 2 46 with acute intestinal obstruction At laparotomy a volvulus of the cæcum and ascending colon was found to have caused strangulation of some 3 to 4 feet of gut The colour of this was very dubious, but became more satisfactory after the application of warm saline packs, and the gut was returned to the abdomen without resection Subsequently paralytic ileus developed and the patient was treated with intravenous saline infusions and continuous gastric aspiration

When seen on 20 2 46 he presented no evidence of dehydration except slight dryness of the mouth , there was on the contrary, generalised œdema involving the whole body except the face and the anterior thoracic wall The abdomen was enormously distended and no peristaltic sounds could be heard B P 140/70

That evening his abdomen was opened again and gross peritonitis was found Cæcostomy was performed and the abdominal cavity drained His condition continued to deteriorate, though his blood pressure remained normal almost to the very end He was febrile during the whole period of observation, the temperature usually ranging between 101° and 102° F He died at 6 a m on 26 2 46

Post-mortem showed necrosis of colon, with perforation in several places , gross peritonitis , subphrenic abscess and R empyema The kidneys appeared normal to the naked eye , microscopically they showed nothing unusual except slight cloudy swelling of the tubules

The results of the investigations are shown in Fig 2 Inulin and diodone clearances were determined on the eighth day after the first operation, and it will be seen that while the diodone clearance was slightly reduced, the inulin clearance was definitely above normal Thus, although the renal circulation was not normal, as is shown by the very high filtration fraction, yet the clearance figures are not sufficiently depressed to explain the high

and rising blood urea (73 mg /100 ml)   It will be noted that on the day of the test the urine volume was 1,800 ml , and the urine urea 3 1 g per 100 ml , this is equivalent to the excretion of 56 g of urea, a grossly abnormal amount   Evidently the cause of the azotæmia at this stage was the large amount of urea which the kidneys were being called upon to excrete, the excess urea production being probably the result of increased protein breakdown associated with fever   However, the blood urea continued to rise, and it may be that another clearance determination towards the end would have revealed impaired renal function, because although the urinary urea concentration remained high, the daily urine volume decreased considerably

The silver nitrate test for urinary chloride was repeatedly negative This would be expected in a case whose plasma chloride was low, but it will be seen that in the present case the value was never below 340 mg /100 ml and rose finally to 390 mg , a figure probably above the upper limit of normal Evidently chloride was being retained in an abnormal fashion by the kidney That plasma base was also being retained is suggested by the terminal rise of plasma CO_2
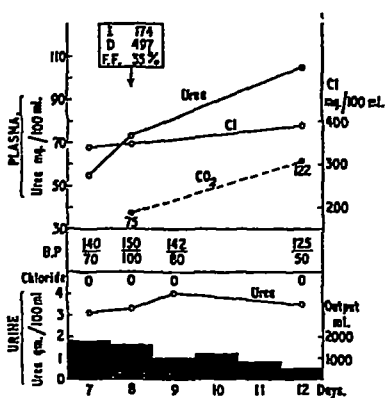
Fig 2

Fig 3

Fig 2   Biochemical findings in Case 3   The zeros opposite ' Chloride ” in the urine section represent results of the qualitative test for urinary chloride with silver nitrate
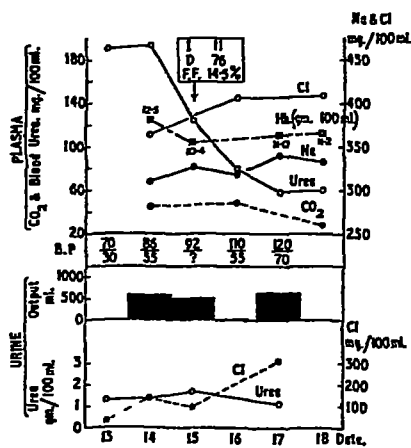
Fig 3   Biochemical findings in Case 4   Figures for hæmoglobin are in g per 100 ml blood

Case 4   A G , female, aged 66   This patient had a cholecystectomy on 27 3 46 for chronic cholecystitis with gall-stones   She was discharged on 1 5 46 with the wound still discharging a little bile

On 16 5 46 she was re-admitted with the fistula still discharging bile The wound broke down on 4 6 46 and the amount of discharge increased By 12 6 46 she was mentally confused, refused her food, and appeared cyanotic and cold , the B P was 70/30

On 13 6 46 she showed gross dehydration of the skin, and the pulse was very feeble   There was no fever   On the following day an intravenous infusion of saline was begun , this resulted in a dramatic clinical improvement, and by 10 a m on 15 6 46, when 3 5 litres saline had been given, the dehydration appeared almost overcome   The patient continued to improve until the night of the 17th-18th, when she had a rigor   The next morning she was semi-conscious and had a fever of 101 2°   She went rapidly downhill and died at 5 a m on the 19th

Post-mortem revealed a gall-stone, about 1 cm in diameter impacted at the lower end of the common bile duct   The peritoneal cavity contained about 230 ml of bile, and early peritonitis was present , there were also many adhesions, of some standing, in the pelvis and round the gall bladder area   The fistula track was not clearly demonstrated, but it appeared to come from the upper end of the dilated bile duct   Rupture into the peritoneum apparently occurred at the junction of the fistula track with the abdominal wall

The kidneys were rather small , the capsules stripped readily, revealing a very faintly granular subcapsular surface   There were several small cysts on the surface   On section, the cortex was pale but the medulla seemed normal   Microscopically there was moderate arteriosclerosis only

The results of the investigations are shown in Fig 3   Inulin and diodone clearances were determined on 15 6 46   The following points may be noted   (1) the clearances show severe renal ischæmia, although the blood urea had already begun to fall   Clinically, there was little dehydration at this point, which suggests that the effects on the kidney of the antecedent period of dehydration may still have been persisting   Subsequently the blood urea fell still further and it seems likely that considerable improvement in renal function took place   (2) the saline infusion produced a fall in hæmoglobin corresponding to an increase in blood volume of 20%, which suggests that the blood volume must have been considerably reduced before treatment   The blood pressure also rose progressively with treatment   (3) the serum sodium on 14 6 46 was 310 mg /100 ml , which suggests some depletion of the plasma base   (4) there was no deficiency of plasma chloride, and *no alkalosis* , in fact the plasma chloride rose and the CO_2 content fell to abnormal levels before the end   This may have been the effect of the large amount of sodium chloride given   (5) the kidneys' power to produce a concentrated urine was impaired , the urine urea never rose above 1 7%

Comment   This patient had evidently suffered a severe loss of fixed base in the biliary discharge, with consequent dehydration and depletion of plasma volume   Whether the reduced arterial pressure was responsible by itself for the renal ischæmia is a matter for speculation, but the reductions in clearance values were so gross as to suggest that active renal vasoconstriction had occurred

Case 5 — E , female, aged 52   This patient had suffered for many years from ulcerative colitis, for which a cæcostomy had been performed 3 years previously at another hospital   The object of the present admission was to close this in the hope of re-establishing normal colonic function The patient's general condition was satisfactory and she complained only of the inconvenience of her cæcostomy

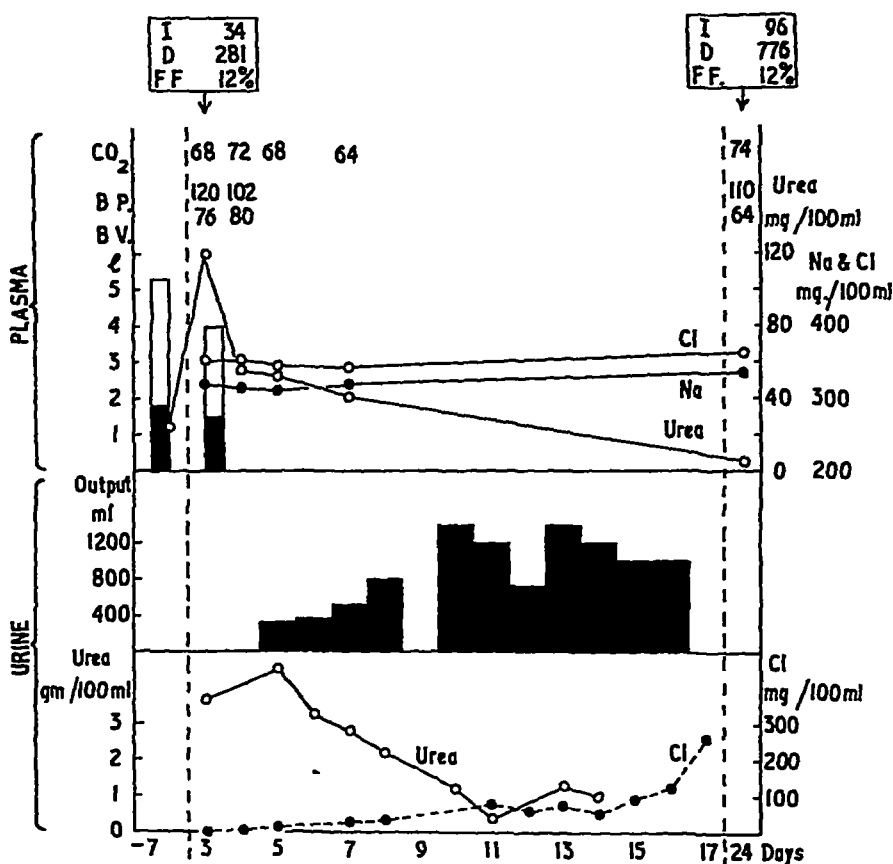Seven days prior to operation her blood urea was 25 mg per 100 ml and her blood volume 5 3 litres

Fig 4   Biochemical findings in Case 5   The time scale is in days after the first operation.

After closure of the cæcostomy she developed symptoms and signs of intestinal obstruction, and on the third day the blood urea was 120 mg /100 ml   (*See* Fig 4)   There was no clinical evidence of dehydration either at this stage or later   However, it will be seen that the clearances revealed gross disturbance, in spite of a normal blood pressure, and a simultaneous determination of blood volume showed that this had decreased to 4 litres

—a loss of 1 3 litres or 25% That evening her abdomen was again opened, and it was found that obstruction had developed immediately distal to the old cæcostomy The cæcostomy was therefore re-established After this the patient made a rapid recovery, the blood urea falling quickly to normal values

The clearances were repeated 24 days after the first operation, with substantially normal results apart from a low filtration fraction The blood volume determination was not repeated as there was still some staining of the skin from the last injection of Evans blue

In this patient, as in Case 2, an unusual relationship between the urinary chloride and the plasma chloride is found The latter remained normal throughout, yet not until nearly a fortnight after the relief of the obstruction did the urine chloride rise above 100 mg /100 ml or the daily chloride output exceed 1 gm

Comment In this uncomplicated case of intestinal obstruction, on the third day considerable reductions in glomerular filtration rate and effective renal plasma flow were associated with a 25% reduction in blood volume, in spite of a normal arterial pressure and absence of clinical dehydration These findings can only be explained on the basis of active vascular change in the kidney, probably vasoconstriction

Discussion

The renal lesion It seems clear from these results that impairment of renal function, sometimes severe, does occur in gravely ill abdominal cases Azotæmia may in certain cases (*e g* Case 3) be mainly due to increased nitrogenous breakdown but in many it is largely or wholly the expression of renal failure While the impairment of diodone excretion might be explained on a functional tubular defect, the changes in inulin clearance cannot be so interpreted, and as in the case of alkalosis the only simple explanation of the overall picture of renal function is that of renal ischæmia

A rather similar renal lesion has been observed previously to follow hæmorrhage, and skeletal injury Thus Black, Powell and Smith (4) investigating 3 cases of gastrointestinal hæmorrhage with azotæmia found that all showed a reduction of renal blood flow as measured by diodone clearance and 2 a diminished glomerular filtration rate In their case 3 in which renal blood flow and glomerular filtration rate were both reduced, the arterial pressure on admission was not subnormal (140/90) The filtration fraction was high in all cases during the phase of ischæmia falling towards normal with recovery Lauson, Bradley and Cournand (17) investigated 35 cases of trauma with varying degrees of peripheral circulatory failure, and found as did Black, Powell and Smith (4) that the clearances often fell much more than could be accounted for by the diminution of arterial pressure , the filtration fraction was variously high,

low or normal   In all these 4 abdominal cases diodone clearance was reduced, slightly in Case 3, moderately in Case 5, and severely in Cases 2 and 4 Accepting the diodone clearance as a measure of blood flow to the renal tubules, it is clear that its reduction must be attributed to vasoconstriction, for the arterial pressure at the time of the clearance determinations was not subnormal in Cases 2, 3 and 5, though a pre-existing hypertension can not be excluded in Case 2   The inulin clearance, measuring the glomerular filtration rate, was reduced in 3 cases, severely in Cases 2 and 4, and moderately in Case 5, and was greater than normal in Case 3   The filtration fraction was high in Cases 2 and 3 (38% and 35% respectively), low in Cases 4 and 5 (14 5% and 12%)   Smith (27) has provided cogent reasons for regarding the filtration fraction as an index of the relative tonus of the afferent and efferent glomerular arterioles, afferent constriction producing a fall and efferent constriction a rise in this fraction   How the filtration fraction would be affected by the intervention of the cortico-medullary shunt mechanism is uncertain , theoretically if the shunt acts by short circuiting blood before it reaches the afferent glomerular arteries, such a shunt should affect inulin and diodone clearance in the same direction, and to the same extent, and should not alter the significance of any deviation from the normal in the filtration fraction   The variability of the filtration fraction in the present series, as in that of Lauson, Bradley and Cournand (17), suggests that in some cases vasoconstriction affected predominantly the afferent, in others the efferent, glomerular arterioles   Phillips and colleagues (23) subjected dogs to graded hæmorrhage and skeletal trauma and found that they tended to show high values for the filtration fraction in the early stages and low values later on   They suggest that when blood volume and cardiac output are reduced efferent constriction is first invoked, its effect being to maintain the glomerular filtration rate in the face of a falling blood flow ,   later this mechanism is overwhelmed by afferent vasoconstriction which tends to cut the kidney out of the circulation altogether and which is analogous to the widespread vasoconstriction affecting the rest of the body   This hypothesis would fit the facts of the present series well enough were it not for one case, No 5   On every other ground this must be reckoned an early case , yet here afferent vasoconstriction appears dominant during the ischæmic phase

In concluding that the kidney in these gravely ill abdominal cases is affected more or less intensely by vasoconstriction, it should be pointed out that the conclusion applies only to the cortex   The observations here described would be perfectly compatible with there being a diversion of blood from cortex to medulla of the kidney

The cause of the renal disturbance   A major difficulty in identifying the cause of the renal disturbance here described is that the lesion may be only slowly reversible, as for example in the case of alkalosis, and as Van Slyke (30) has shown for the kidney of the dog after temporary complete

ischæmia Thus in a given case, the renal picture may be related causally not so much to present as to past changes Nevertheless in view of its importance some attempt may be made to define the problem In these cases loss of blood, the predominant factor in the series of Black, Powell and Smith (4) and of Lauson, Bradley and Cournand (17) can be largely or entirely excluded On the other hand a reduction in blood volume was demonstrated in Case 5, inferred in Case 4 and probable in Case 2 Clinical signs of dehydration were present initially in Case 4 which had the most severe renal ischæmia, absent in Case 5 where renal ischæmia was moderate, and in Case 3 where renal ischæmia was least and glomerular filtration supernormal, excessive hydration had been produced On the present evidence then dehydration would seem to be at least one possible major cause of the renal lesion and may be suspected of operating through a reduction in blood volume and cardiac output with consequent widespread vasoconstriction Alkalosis is another possible factor in abdominal cases treated by gastric suction or alkalis to prevent sulphonamide crystals in the urine, or where vomiting is a conspicuous feature , but alkalosis was absent in these cases

The role of tissue injury and peritoneal infection is more difficult to assess At the outset it seemed possible that these might prove major causative agents This, however, seems unlikely in view of the results in Case 3 where extensive bowel necrosis and peritonitis were demonstrated at operation on the day before clearance tests revealed only slight renal ischæmia It is clear that much more work will be needed to solve this problem and for the moment it is enough to stress that the present work suggests the importance of combating dehydration by replacement of water and salt if gross renal impairment is to be prevented

Chloride retention A point requiring further consideration is the chloride retention found in Cases 2 and 4 and 5 This is by no means a new finding Allott (1) described 5 similar cases, 4 of which had an intracranial lesion, and Cooke (8) observed chloride retention in some of his alkalosis cases, often lasting 4-8 days after the plasma chloride had reached a normal level If we accept the theory that chloride is normally excreted in the urine whenever it is above a certain threshold, usually about 95 mEq/l (337 mg /100 ml) (Allott (2)), the findings recorded here can only mean that the threshold has been set at an abnormally high level in these cases The mechanism of this is not clear It is not related to the renal failure, since Case 3 had chloride retention but nearly normal clearances, while Case 4 had renal failure but no true chloride retention Allott's (1) cases showed minimum excretion of sodium and enormously increased excretion of potassium, with raised sodium and lowered potassium in the serum , the hypothesis that an increased secretion of adrenal cortical hormone was responsible is a very attractive one Case 5 in the present series showed a slight rise in serum sodium, but no potassium determinations were *done*

Final Comment This work was prompted by the continued high death rate in battle casualties with abdominal wounds The evidence presented shows that gross changes in renal function occur in abdominal cases of civilian surgical practice and suggest that the changes are due to renal ischæmia It would, however, be a grave mistake to suppose that the kidney is the only organ affected in such cases, though it happens to be a convenient one for investigation Renal ischæmia is most probably only part of a widespread circulatory disturbance, a disturbance which if sufficiently severe causes death

SUMMARY

One case of alkali alkalosis and four of abdominal catastrophe all showing a raised blood urea have been investigated with the inulin and diodone clearance techniques The case of alkalosis was remarkable in that initially clinical dehydration was associated with polyuria , the tests showed grossly reduced glomerular filtration rate and effective renal plasma flow , changes which recovered only slowly and incompletely after restoration of the acid-base balance of the blood

The four abdominal cases showed in general the picture of renal ischæmia which varied from mild to severe The ischæmia is attributed to cortical vasoconstriction but no evidence relevant to the possibility of a cortico-medullary shunt has been obtained The chief factor in the causation of the renal lesion in this series appears to be dehydration It is probable that renal ischæmia is only part of a general disturbance of the circulation in such cases, a disturbance which up to the present has not been investigated

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BLOOD CHOLATES IN NORMAL SUBJECTS AND IN LIVER DISEASE

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BILE salt metabolism has been investigated less than any other hepatic function   This has been largely due to the poor analytical methods existing for the determination of bile acids in biological fluids   The development of a more satisfactory technique for the estimation of blood cholates (7) stimulated us to study the cholate values in the blood of normal subjects and in those suffering from hepatic disease   The purposes of the work were firstly the search for a procedure possibly useful in the diagnosis of mechanical obstruction from parenchymatous jaundice and secondly the elucidation of the mechanism of the changes in blood cholic acid concentration by comparing results obtained with the histological appearances in liver biopsy sections (21)

Clinical material

In all, 50 normal subjects and 110 patients with disease involving the liver have been studied

Normal subjects were usually members of the hospital staff   A few patients convalescent from non-hepatic diseases were included   The serum bilirubin concentration in every subject was less than 1 0 mg /100 ml

Acute hepatitis (50 cases)   This group includes simple infective hepatitis (29 cases), jaundice following plasma infusions (4 cases) and jaundice occurring during the course of arsenical therapy for syphilis (17 cases) In 30 of these patients one or more aspiration hepatic biopsies were performed   The biopsy material has been grouped according to the extent of liver cell damage in order to compare the histological changes with the chemical findings (Fig 3)   The method of biopsy has been described previously (22)

Cirrhosis of the liver (20 cases) The diagnosis in each patient was confirmed by study of hepatic histology Two groups, "active" and "latent" were recognised (22)

Obstructive jaundice (21 cases) In every instance there was complete obstruction of the common bile duct confirmed at operation or autopsy

Intermittent obstructive jaundice (6 cases) This group included three patients with gallstones and single examples of xanthomatous biliary cirrhosis, traumatic stricture of the common bile duct, and carcinoma of the ampulla of Vater There was varying stool colour with fluctuating presence and absence of urobilinogen in the urine Urobilinogen was present in the urine at the time of withdrawal of blood for the cholate estimation

Secondary hepatic malignant disease (7 cases) In every instance the diagnosis and the extent of hepatic involvement were confirmed by autopsy Cases with jaundice due to occlusion of the common bile duct were excluded

Haemolytic jaundice (6 cases) In every instance a constant reticulocytosis was demonstrated in the peripheral blood and there was urobilinuria and hepatic siderosis

Methods

Estimation of blood cholates A modification of Josephson's (1935) method was used This is based on the reaction in which a blue colour is obtained when bile salts are heated with 45-50 per cent v/v sulphuric acid and a dilute solution of furfural (4) Accurate results can only be obtained if pure reagents are used and all interfering substances are first removed from the blood 10 ml whole blood are withdrawn from an antecubital vein and immediately pipetted with shaking into an ethanol-ether mixture (60-40) containing saturated barium hydroxide (5 ml) With this protein precipitant, the mixture can be filtered after 30 minutes, whereas 12 hours' standing is necessary if pure ethanol is used The excess barium is precipitated with a single drop of 5 N sulphuric acid and the pH brought back to 9 0 with a drop of 6 N sodium hydroxide A volume correction is thus avoided The extraction of interfering substances is carried out as in Josephson's method The final solution is filtered while still warm, through a Whatman 45 paper The occasional turbid sample can be cleared by adding one or two drops of glacial acetic acid 2 5 ml filtrate (equivalent to 2 0 ml blood) are pipetted into a pyrex tube and sulphuric acid (5 0 ml 50% v/v) and aqueous furfural (0 5 ml 2%) added The furfural is redistilled immediately before making up the 2% solution The contents of the tubes are well mixed and heated in a water bath at 65° $\pm$ 1° for 15 minutes The blue colour is then maximal The colours are read in a photo-electric colorimeter, using a Chance Red filter and against a reagent blank prepared by substituting water for blood filtrate Pure cholic acid (Hopkins and Williams) was used as the standard and results were read off from a calibration curve constructed for the colorimeter used

The results are expressed as mg sodium cholate per 100 ml blood The recovery of cholates from whole blood has been checked by a number of analyses, many in duplicate (Fig 1)
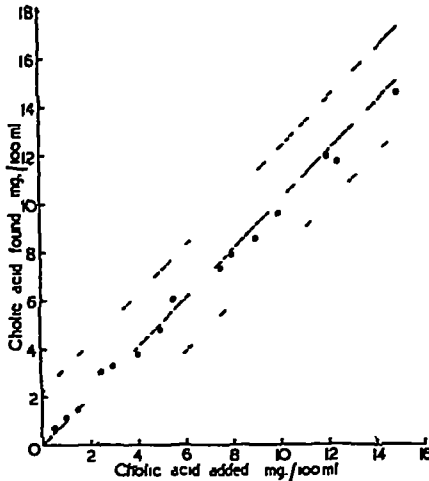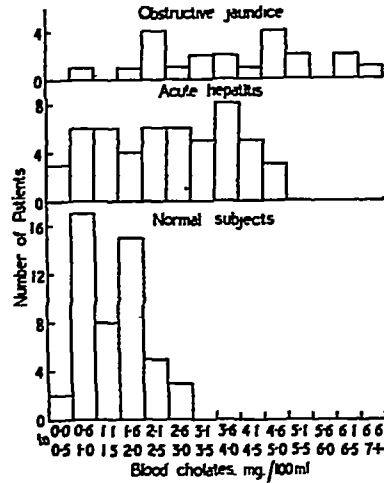

Fig 1

Fig 2

Fig 1 The recovery of added cholate from normal blood The dotted lines represent the range ± 3 S D where S.D is the standard deviation of the difference between cholic acid found and that added S.D = 0 51 mg per 100 ml

Fig 2 Histograms of the frequency distribution of blood cholates in normal subjects, acute hepatitis and obstructive jaundice

General biochemical methods The estimations carried out were the serum bilirubin (5), alkaline phosphatase (13), total cholesterol (20), together with the total and differential serum proteins (14, 15)

Results

The results of the complete series are summarised in Table I

Normal subjects The blood cholate concentration lay between 0 2 and 3 0 mg/100 ml (Fig 2)

In 5 normal subjects the blood cholate concentration was estimated at weekly intervals for 9 weeks (Table II) Simultaneous serum bilirubin estimations in 4 of the subjects were always below 0 5 mg /100 ml , in one it fluctuated between 0 5 and 1 2, the changes occurring independently of changes in the blood cholate values In three normal subjects the blood cholate concentration was estimated at intervals during the day (Table III) The diurnal variation is slight, inconstant and unrelated to absorption from the intestine

TABLE I

Biochemical results in 110 cases of liver disease

Throughout this table, figures in normal type are mean values, figures in italics are standard errors of mean values, and figures in brackets are observed ranges of variation of results

	No of cases	Blood cholates mg /100 ml	Serum bilirubin mg /100 ml	Serum phosphatase units /100ml	Serum cholesterol mg /100 ml	Serum proteins g /100 ml Total	Albumin	Globulin
Normal	50	1 55 / *0 012* / (0 2–3 0)	(0 5–1 0)	(4–13)	(120–230)	(6–8)	(3 4–5)	(1 5–3 0)
Acute hepatitis	50	2 76 / *0 19* / (0 5–5 4)	6 91 / *0 60* / (1 0–18)	17 / *0 99* / (4–35)	217 / *5 05* / (111–327)	6 08 / *0 13* / (5 6–9 5)	3 97 / *0 081* / (2 7–5 2)	3 01 / *0 080* / (1 7–4 6)
Hepatic cirrhosis Active	14	1 96 / *0 24* / (0 5–4 0)	4 03 / *0 94* / (0 9–12 2)	14 9 / *1 34* / (7–27)	192 3 / *8 65* / (115–361)	6 5 / *0 64* / (5 1–8 1)	3 0 / *0 15* / (2 0–4 3)	3 5 / *0 33* / (2 8–4 4)
Latent	6	1 41 / (0 5–2 0)	0 63 / (0 5–0 8)	13 4 / (4–24)	182 / (167–195)	6 6 / (5 9–7 0)	4 3 / (3 1–5 2)	2 3 / (1 1–2 8)
Obstructive jaundice Complete	21	4 0 / *0 37* / (1–8 0)	11 9 / *1 3* / (4–27)	47 / *6 3* / (20–136)	210 / *4 12* / (121–600)	6 6 / *0 16* / (5 0–7 7)	3 7 / *0 12* / (2 7–4 7)	2 9 / *0 13* / (1 8–4 2)
Intermittent	6	1 41 / (0 8–2 2)	5 3 / (1 9–13)	36 / (25–120)	364 / (153 1160)	6 3 / (5 9–9)	3 6 / (3 1–4 2)	3 7 / (2 6–4 4)
Haemolytic jaundice	6	1 47 / (0 2–3 0)	1 6 / (0 5–2 9)	10 7 / (4–22)	145 / (127 181)	6 8 / (6 4–7 1)	4 4 / (3 6–4 9)	2 4 / (1 6–3 4)
Secondary malignant	7	2 1 / (1 0–3 0)	0 83 / (0 5–1 8)	14 6 / (5–3 2)	184 / (151–243)	5 8 / (5 4–6 7)	3 5 / (2 9–4 4)	2 3 / (1 0–3 0)

TABLE II

Resting levels of blood cholates in 5 normal subjects over a period of 9 weeks (mg /100 ml)

Subject	Sex	Weeks 1	2	3	4	5	6	7	8	9
P	F	2 0	2 2	2 0	1 0	1 0	3 0	0 0	1 0	1 5
L	F	2 5	2 5	0 0	1 5	1 0	2 5	0 0	1 5	0 9
H	M	1 1	0 5	1 2	0 5	—	0 8	2 0	0 6	1 8
T	M	0 75	2 0	1 5	0 0	1 0	0 5	1 0	0 9	2 0
W	F	0 75	1 0	1 2	1 5	1 5	1 1	2 0	1 5	1 5

TABLE III

Blood cholates, (mg /100 ml)

Subject	Sex	8 a.m	12 noon	4 p.m.	12 midnight
W	F	0 7	0·6	0 5	0 9
H	M	0 4	0 7	0 4	0 8
B	M	0 5	0 7	0 4	0 8

Variation in blood cholates in 3 normal subjects
Meals were taken at 8 15 a.m   1 p.m   4 15 p.m   8 p.m.

Acute hepatitis   The results obtained were much more variable (Fig 1) and the mean value was higher than for normal subjects   A significant positive correlation existed between the serum bilirubin and blood cholate concentrations   There was no correlation with serum alkaline phosphatase, cholesterol or the serum proteins

Hepatic histological sections were available in 30 instances   Comparison of the extent of hepatic cell damage with the blood cholate values showed that, although there is an increase of doubtful significance in the mean values recorded in grades A and B, there is no significant change in the mean of the three more severe grades (Fig 3)

Grade	A	B	C	D
Probable percentage surviving liver cells	75-100	50-75	25-50	< 25
Mean blood cholate level mg /100ml	2 0	2 8	2 6	3 1
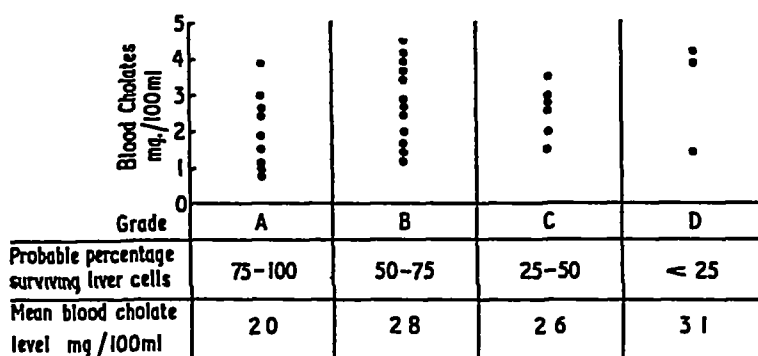
Fig 3   Acute hepatitis   the blood cholate values in the histological grades of increasing severity from A to D

The level is highest early in the disease, falling with recovery   This is shown when the patients are grouped into those early in the icteric phase with rising serum bilirubin concentration and urobilinogen absent from the urine, and those later in the icteric phase with falling serum bilirubin and urobilinogen present in the urine (Table IV)   A significantly higher mean blood cholate concentration occurs in the first group   The mean serum bilirubin concentration and duration of jaundice are not significantly different in the two groups

TABLE IV

Blood cholic acid in acute hepatitis at the stage of increasing and diminishing jaundice

Figures give mean values with the range of observations indicated in brackets

Stage of disease	No of cases	Mean duration jaundice (days)	Mean serum bilirubin mg /100 ml	Mean blood cholates mg /100 ml
Rising serum bilirubin Urobilinogen absent in urine	21	12 (32–1)	9 1 (17 4–2 8)	3 8 (5 4–0 0)
Falling serum bilirubin Urobilinogen present in urine	29	13 (35–2)	6 1 (18–1 6)	2 03 (4 5–0 5)
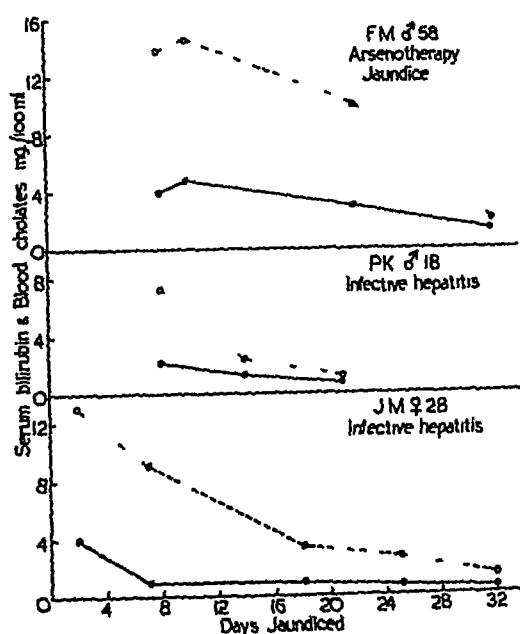
Fig 4
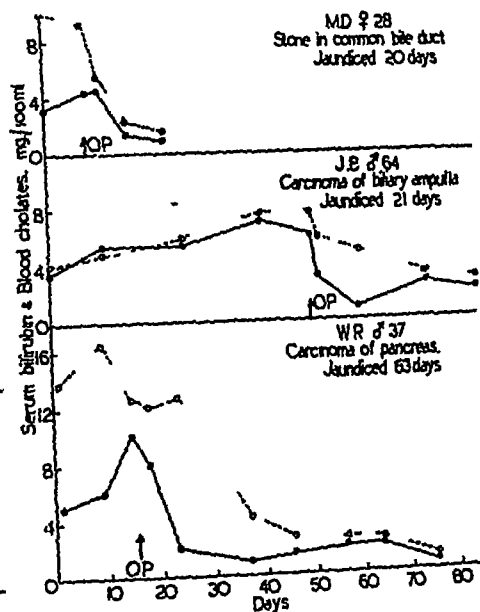
Fig 5

Fig 4   Acute hepatitis   the course of the serum bilirubin and blood cholates Interrupted line serum bilirubin, continuous line blood cholates

Fig 5   Obstructive jaundice   the course of the serum bilirubin and blood cholates Interrupted line serum bilirubin, continuous line blood cholates   The duration of jaundice on admission to hospital and the time of surgical relief of the obstruction (Op) are indicated

The course of the blood cholates in 3 patients is illustrated (Fig 4) The levels recorded on admission to hospital are usually the highest In only 2 of 28 patients did serial blood cholate estimations show an increase after the first week in hospital In these 28 patients the blood cholate was followed at weekly intervals until the serum bilirubin concentration had

fallen below 2 mg /100 ml   Even though the blood cholate value initially
is often within normal limits, with recovery there is a decrease to a lower
normal value   The blood cholate concentration fell in 27 of the 28 patients
The change in the mean value before and after recovery is statistically
significant (Table V)

TABLE V

*Acute hepatitis   the serum bilirubin and blood cholate concentration before and after recovery
in 28 cases*

	Mean duration of jaundice (days)	Serum bilirubin mg /100 mL.		Blood cholates mg /100 mL.	
		Mean	S E of mean	Mean	S.E of mean
Acute stage	10	7 29	0 73	3 01	0 25
After recovery	29	1 45	0 07	1 31	0 14

With recovery from hepatitis the fall in the blood cholate concentration
is very rapid (Fig 4)   The lowest level recorded for that individual was
reached 7 to 14 days before the serum bilirubin had reached 2 mg /100 ml

Obstructive jaundice   The results obtained were much more variable
and the mean value was higher than that for both acute hepatitis and for
normal subjects (Fig 2)   Statistical analysis is not very helpful on such a
small series, but there does seem to be a positive correlation between the
serum bilirubin and blood cholate concentrations   There is no correlation
between serum alkaline phosphatase, cholesterol or serum proteins and blood
cholates   The course of the blood cholate concentration in 3 patients is
illustrated (Fig 5)   When there was histological evidence of increasing
hepatic damage the cholate concentration progressively rose during the
course of obstructive jaundice   As late as the 69th and 78th day of jaundice
the value was still rising in the blood   In six patients the level was followed
during the illness ,   in no instance was there any spontaneous falling off
of the blood cholate concentration   In five of these patients surgical relief
of the biliary obstruction was possible   The blood cholate concentration
fell to normal very rapidly   Normality was reached before the serum bili-
rubin or alkaline phosphatase concentrations approached normal

Intermittent obstruction   Even though all the patients were jaundiced
and showed a raised serum phosphatase concentration the blood cholate
values were normal

Hepatic cirrhosis   Normal blood cholate concentrations were found
in all the patients in whom activity of the cirrhosis (22) could not be shown
by other biochemical methods or by study of hepatic histology   In the
active group there was a wide variation, and although the mean fell within

the normal range, two patients showed values above the upper limit for normal subjects These patients were not the most severely ill or the most deeply jaundiced

In hepatic cirrhosis, whether active or latent, a positive correlation could not be established between the serum bilirubin and blood cholate values

Secondary hepatic malignant disease and haemolytic jaundice   The blood cholate concentrations were normal (Table I)

Discussion

The wide differences in the levels of bile acids reported in the blood of normal subjects—from none at all (16, 18, 29) to 100 mg /100 ml (27) have been noted—may be attributed to the various analytical methods used Many were unspecific and interference by cholesterol and lipoids makes the results unreliable Moreover, normal values have often been quoted on a very small number of observations Workers using methods similar to ours have reported similar results (19, 7) These methods estimate cholates and glyco-taurocholates but not deoxycholic acid It is unlikely that the latter substance occurs in appreciable quantities in normal blood Apart from some nocturnal decrease there is probably no rhythm in cholate production by the liver (11) Large and unphysiological quantities of bile salts introduced into the intestine of experimental animals produced only a very slight rise in the systemic blood cholates (8) It is not surprising, therefore, that the continuous elimination into the intestine of the normal amount of bile salts fails to produce any diurnal variation in the systemic blood cholate values

Bollman and Mann (1) found that in dogs bile salts were not present in the blood or urine after complete hepatectomy, and injected bile salts were quantitatively recovered in the urine After operative exclusion of the liver in cats and rabbits there was a fall in blood bile salts (12) This suggests the hepatic formation of bile salts Moreover, in experimental animals the formation of bile salts is inhibited by the administration of hepatic poisons such as chloroform and carbon tetrachloride (1, 25) In hepatitis in man, however, the blood cholic acid concentration is usually increased (10, 28), an observation confirmed in the present series However, the expected fall with increasing liver damage in hepatitis was not recorded The discrepancy between these findings in man and experimental animals may be associated with the differences in the hepatic pathology In experimental hepatic poisoning the liver cells are most affected, the intrahepatic biliary tree is relatively intact, whereas in infective hepatitis there is not only hepatic cell necrosis but also disruption of the liver cell columns with their associated intercellular bile canaliculi (3) There is therefore not only possibly *diminished production* of bile salts by diseased liver cells but also an interruption of the channel for their excretion The occurrence of the highest blood bile salt concentrations at the phase when urobilin is absent from the

urine and the serum bilirubin concentration is rising supports this conception Serial hepatic biopsies have demonstrated the very rapid recovery which usually occurs in acute hepatitis The bile channels are quickly restored This coincides with the rapid fall of the blood cholate level

The blood cholate concentration in cirrhosis is very variable (10, 19) Normal values have been recorded (26) The variability may be associated with the complex histological picture of hepatic cirrhosis Diminution of the absolute number of liver cells will lessen the production of bile salts the vascular tree of the cirrhotic liver is reduced (17) with diminution of the amount of blood supplied to the surviving parenchyma distortion of the biliary tree may impede the excretion of the bile salts that are formed In hepatic cirrhosis the balance between these three factors, and possibly others, probably determines the variable level of blood cholates

The changes in blood cholates recorded in obstructive jaundice have a simpler pathogenesis than those in acute hepatitis or hepatic cirrhosis The rise usually recorded is due to a simple retention in the blood of bile salts manufactured by the hepatic cells the exit of which is prevented by the biliary obstruction The level is said to fall in the later stages of the disease (6, 19) , this is usually attributed to diminished output of bile salts by the liver cells which are known to become increasingly damaged by prolonged obstruction (23) In the present series, however, the blood cholate concentration has not diminished in the later stages of unrelieved obstruction A rising blood cholate concentration has been observed for as long as three months in several patients suffering from unrelieved obstructive jaundice Apparently in these cases the hepatic damage produced by biliary obstruction was not of sufficient severity to completely prevent cholic acid production

During recovery from acute hepatitis and following relief of a mechanical obstruction, the concentration of cholates in the blood returns to normal much more rapidly than does the serum bilirubin value The slow fall in serum bilirubin is often attributed to combinations with tissue protein from which the pigment is but slowly dissociated Bile acids are absorbed on to vascular endothelium (9), and in obstructive jaundice accumulations have been shown in liver, muscle and kidney (2) The excretion of bile acids would be expected therefore to be slow It may be, however, that bile acids are excreted in the bile more readily than bilirubin. This suggestion is supported by the rate of disappearance of the two substances after intravenous injection, that for cholates being much more rapid than that for pigment (24)

The liver functional changes in haemolytic icterus are minimal, and it is not surprising that the blood cholates values are normal Similarly, in the case of malignant hepatic metastases there is usually adequate remaining liver tissue to maintain a normal blood cholate concentration

The bile acids are probably manufactured solely by the liver, whereas serum bilirubin, alkaline phosphatase and proteins are partially formed outside the liver and are not influenced to the same extent by factors altering the production and excretion of bile acids   The difficulty in demonstrating in liver diseases a strongly positive correlation between blood cholates and these other substances is therefore not unexpected   As bile acids are probably formed only by the hepatic cells it is regrettable that changes in blood cholate are not more frequent in the presence of organic liver disease   Blood cholate values are in fact an insensitive index of hepatic dysfunction and have not proved to be the long-sought estimation that will demonstrate the lesser degrees of hepatic damage

The frequently high blood cholate concentration in jaundiced subjects, often higher in obstructive jaundice than in acute hepatitis, suggests the estimation might have a diagnostic application   This is not the case (Fig 6)   Not only is there much overlap of the values for acute hepatitis and obstructive jaundice, but 7 of 21 patients with obstructive jaundice and 30 of 50 with acute hepatitis have blood cholate concentrations below the upper limit for normal subjects
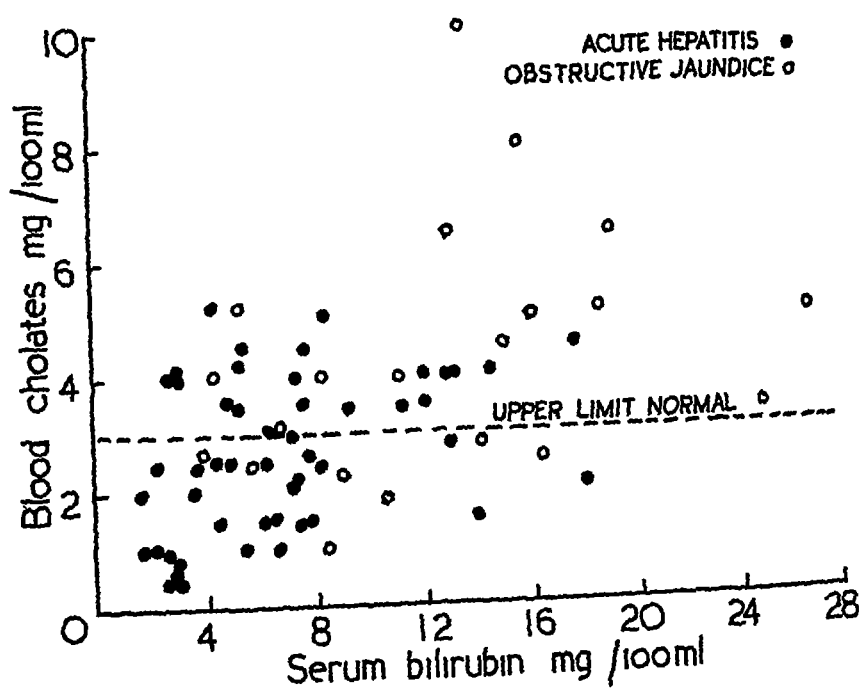
Fig 6   Serum bilirubin and blood cholates in acute hepatitis and obstructive jaundice

Summary

1   Blood cholate has been estimated in 50 normal subjects and in 110 patients with hepatic disease and the results have been compared with the concentrations of serum bilirubin, alkaline phosphatase, cholesterol,

albumin and globulin, and with the histological appearances of sections of liver obtained by aspiration biopsy

2   In normal subjects there was no significant diurnal or weekly variation in the blood cholate values

3   In acute hepatitis the mean of blood cholates was raised, but results were variable   Values did not significantly diminish in the patients showing the more severe degrees of hepatic damage

4   In acute hepatitis blood cholates are highest during the phase of rising serum bilirubin and absence of urobilinogen from the urine   There is a rapid fall with recovery

5   In obstructive jaundice values are again variable   The highest values are recorded in this group   The blood cholate concentration progressively increased during the course of the obstruction   Relief of biliary obstruction results in rapid fall to normal of the blood cholates

6   In both acute hepatitis and obstructive jaundice a moderately significant correlation existed between blood cholates and serum bilirubin There was no relation between the values for blood cholates and serum alkaline phosphatase, total cholesterol or proteins

7   In hepatic cirrhosis, whether active or latent, the blood cholates were usually normal

8   In hæmolytic icterus and secondary malignant disease the blood cholate levels were normal

9   Blood cholate values are of no value in the differential diagnosis of different forms of liver disease nor in the detection of minor degrees of hepatic dysfunction

10   The mechanisms of the changes in blood cholates in liver diseases are discussed

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THE INTRALOBULAR CIRCULATION IN ACUTE LIVER INJURY BY CARBON TETRACHLORIDE

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A LARGE variety of poisonous substances, of which carbon tetrachloride and chloroform are examples, are especially toxic to the liver Directly applied to the organ they cause immediately a thin layer of coagulative necrosis of the hepatic parenchymal cells and, when high concentrations are produced in the hepatic blood as by directly injecting such poisons into the portal vein (2), a similar immediate necrosis appears throughout the lobules of the areas affected When, however, the liver cells are exposed to lower concentrations, as occurs when the poisons are given by the less artificial means of inhalation, ingestion or subcutaneous injection a different type of damage results Necrosis still develops in the parenchyma but it is neither immediate, nor coagulative, nor widespread in the lobule On the contrary its appearance is delayed for several hours , its morphological form is hydropic , it is limited precisely to the zone of parenchyma around the central veins This is the type of necrosis which is seen in man and it is with this type that we are concerned at present

Cameron, Karunaratne and Thomas (3), using intraportal injections of carbon tetrachloride, showed that the liver rapidly removed this substance from the blood for they found that the animal would tolerate several times the lethal dose, as determined by injection into a systemic vein, if this were given directly into the portal vein This ability to absorb the poison is largely, or even exclusively, a property of the parenchyma for it alone is damaged Further it is a general property of hepatic parenchymal cells, and not limited to those in a particular zone, for, after intraportal injection, the cells in all zones of the lobule are affected At first sight, the centrilobular necrosis following the lower, and more usual, concentrations of the poison within the liver might seem to imply that the blood, and with it the poison, was concentrated in its passage down the hepatic sinusoids to such an extent that the concentration of the poison reached levels sufficient to kill the hepatic parenchyma But three observations render this explanation untenable The centrilobular necrosis does not appear until eight to twelve

hours after exposure   Morphologically it is of a different type from that produced by the direct action of the poison   The rate of blood flow through the liver is so large (1) that volumes of lymph and bile, far exceeding those ever observed, would have to be removed to concentrate the poison to necrotising levels   We are thus faced with the paradoxical observation that, when poisons like carbon tetrachloride are given by mouth, by inhalation or by subcutaneous injection, the resulting necroses do not occur where the concentration of the poison is presumably highest, namely in the periportal zones, but where it is presumably lowest, namely around the central veins This well established observation is explicable in one of two broad ways, either the local conditions at the centre of the lobule are such as greatly to increase the susceptibility of the parenchymal cells to injury by toxins, or the centrilobular necrosis is attributable to some other mechanism than a direct action of the poison on the parenchyma   From the close morphological similarity between the centrilobular zonal lesion produced by certain liver poisons and that seen after impairment or arrest of the hepatic circulation, the possibility arises that in both instances a similar mechanism is at work, namely circulatory insufficiency   It was, therefore, decided to investigate the effect of carbon tetrachloride upon the intrahepatic circulation

Methods

The state of the intrahepatic circulation was visualised by injection of dye   Rats were anæsthetised with ether and the liver and spleen exposed by a midline abdominal incision   0 75—1 0 ml of freshly filtered Mandarin black* was injected slowly into the spleen through a fine needle, the injection taking 60 seconds   The circulation was then immediately arrested by clamping the aorta and inferior vena cava just above the diaphragm   The vessels in the portal fissure were ligated, the liver removed and suspended, with its vessels still ligated, in 10 per cent formol saline   After two hours fixation thin pieces were cut and returned to the fixative for a further twenty-four hours

Albino rats of the Wistar strain weighing between 180 and 250 gms were used   They were kept for several days on the laboratory stock diet, but were not fed on the morning of the experiment   Water was provided in unrestricted amounts   Carbon tetrachloride (Analar) was injected subcutaneously in a dose of 0 2 ml per 100 grammes of body weight   At intervals of approximately 2, 4, 8, 12, 18 and 24 hours the state of the intrahepatic circulation was determined by the technique described above

Control animals, which had received no previous injection of carbon tetrachloride, were studied in the same way

* A preparation of waterproof carbon ink made by Winsor and Newton, Ltd, London, England   The particle size in a filtrate through Whatman's No 1 paper does not exceed 0 5μ

Sections were cut at 15μ by the freezing technique and stained with Ehrlich's acid hæmatoxylin   After paraffin embedding, sections cut at 6μ were stained with hæmatoxylin and eosin

Results

Macroscopic   In controls the ink flowed rapidly through the liver and lungs and into the systemic arteries   On beginning the injection the left half of the liver was, in almost all cases, well injected before much dye flowed into the right half, but, as the injection continued, all lobes of the liver became blackened   At first the injection flowed more readily into some areas than into others, even in the same lobe, but, at the end of one minute, the liver was uniformly black

Two hours after injection of carbon tetrachloride, the entrance of the dye into the liver was slower than normal but otherwise showed no new feature

Four hours after injection of carbon tetrachloride, the dye flowed slowly into the liver, sharply revealing a reticular pattern instead of smoothly blackening the whole organ, as in the earlier specimens

Eight hours after injection of carbon tetrachloride, the greater part of the liver showed the reticular pattern without diffuse blackening, but intense blackening of the liver occurred in a few small irregular patches

Twelve, eighteen and twenty-four hours after injection of carbon tetrachloride, the entrance of the dye was slow   By the end of 60 seconds it had diffused fairly uniformly through the whole liver, but small irregular patches were intensely injected

Microscopic   Sections of liver from the control animals showed uniform filling of the sinusoids which were well injected from the portal tracts to the central veins (Fig 1)   Two hours after carbon tetrachloride there was definite impairment of blood flow as shown by the poor and irregular penetration of the sinusoids   In some lobules the injection had failed to penetrate even into the periportal sinusoids and in the majority had failed to reach the central zones (Fig 2)   The hepatic cells throughout the lobule appeared swollen and in the centrilobular zone fine vacuoles were present in the parenchyma so that it stained more lightly   At four hours (Fig 3) the impairment of the sinusoidal circulation, as estimated by the penetration of the injection, was even more pronounced than at two hours and the change in the central cells was more conspicuous   At eight hours hydropic degeneration of the central cells was already apparent   Closure of the sinusoids was still a striking feature, but it lacked the uniformity shown at four hours, penetration of the ink having occurred in a few irregularly distributed areas   At twelve and twenty-four hours (Fig 4) centrilobular zonal hydropic degeneration and necrosis were severe   In the majority of

the lobules the injection was only present in the portal veins and peripheral sinusoids In a small minority, however, the circulation was apparently becoming re-established

It is thus apparent that a great restriction of the intralobular circulation occurs soon after administration of carbon tetrachloride, and persists for many hours Degenerative changes in the parenchyma, however, are not apparent until some hours after the appearance of the circulatory restriction

Discussion

The effect of carbon tetrachloride on the intrahepatic circulation has been studied *in vivo* by Wakim and Mann (24), using the quartz rod technique of Knisely (13, 14) On momentarily holding a swab soaked in carbon tetrachloride to the nose of a rat under urethane anæsthesia they observed an immediate, but transient, constriction of the sinusoids Rats exposed to carbon tetrachloride vapour for periods as long as thirty minutes showed severe and persistent reduction of the intralobular circulation with obliteration of many sinusoids In these, twenty to twenty-four hours after exposure, most of the sinusoids were obliterated, the majority of those still patent being supplied by the hepatic artery Two to four weeks later the appearance of the liver and its circulation had returned to normal Subcutaneous injection of 0 1 ml of carbon tetrachloride produced similar changes Using a similar technique, Loeffler and Nordmann (16) had previously observed a sharp narrowing of the sinusoids in mice and rats within fifteen minutes of inhalation of chloroform, the portal veins meanwhile remaining quite patent These changes persisted for several hours After ten to twenty-four hours the centrilobular sinusoids dilated but the blood flow was still retarded Return to normal was gradual, taking from three to ten days

These results of direct *in vivo* microscopy are therefore in general agreement with those obtained by *in vivo* injection There is, however, some discrepancy in the time relationships This is probably due to the less physiological nature of the injection method, which might itself seriously affect the circulation Wakim (22) has indeed shown that the normal intermittent type of flow, in which 75% of the sinusoids are inactive (Wakim and Mann, (23)), changed to 100% activity within a few minutes of an intravenous injection of Trypan blue or Indian ink The patchy injection of the liver observed in the first few seconds following intrasplenic injection of Mandarin black and the subsequent rapid diffusion, more or less uniformly, into the rest of the liver is no doubt the visible expression of this effect The final injection picture in any individual case is therefore the resultant of the dilating effect of the ink and the constricting effect of the carbon tetrachloride This probably explains why in our experiments relatively good injections were obtained at a time when, according to the results of direct microscopy, the sinusoids should still have been tightly constricted

It having been established by *in vivo* microscopy and by injection that the hepatic circulation is seriously impaired following exposure to carbon tetrachloride, it may well be asked to what extent does this circulatory impairment contribute to the production of the liver lesions characteristic of carbon tetrachloride poisoning? Extreme impairment of the intralobular circulation can be produced by ligation of the vessels, but the results of ligation of the hepatic artery or of the portal vein vary in different species and with the circulatory state of the individual animal (19) In all cases, however, where both vessels are ligated, necrosis occurs rapidly (15), even when the hepatic artery alone is ligated necroses may be macroscopically visible after four hours (4) and are always marked after twelve hours The impairment of the hepatic circulation induced by carbon tetrachloride persists for at least eight hours and during the whole of this period the intralobular parenchyma is relatively ischæmic Ligation of the large vessels supplying the liver shows that ischæmia is most severely felt in the central zone of each lobule (4, 19) The development of a similar centrilobular necrosis, occurring at the expected time after an ischæmia has been induced by carbon tetrachloride, indicates the possibility of a common basis It is suggested, therefore, that the centrilobular necrosis produced by carbon tetrachloride is the expression, not of a direct toxic action of that poison, but of an ischæmia of the centrilobular region

It is not yet possible to attribute the necrosis of the liver cells consequent upon ischæmia to the deprivation of any one of the innumerable blood constituents But two, oxygen and the sulphur-containing amino-acids, deserve special consideration Anoxia alone can cause the degeneration of the central liver cells (17) The lesions produced in hyperthyroid animals exposed to low oxygen tensions for several hours are indistinguishable from the zonal necroses produced by carbon tetrachloride (18) Oxygen protects the liver against injury by chloroform (8) or carbon tetrachloride (7) Moreover the figures for oxygen saturation of the portal and hepatic vein blood during chloroform anæsthesia obtained by Goldschmidt, Ravdin and Lucké (8) indicate a considerable degree of hepatic anoxæmia Finally, the first evidence of anoxia is a fine vacuolation within the parenchymal cells and, when anoxia persists, this progresses to hydropic degeneration (21) Exactly the same sequence of morphological changes is noted in the centrilobular cells after exposure to carbon tetrachloride The sulphur-containing amino-acids, cystine and methionine, also play an essential part in maintaining the integrity of hepatic parenchymal cells Dietary deficiency of these substances, if maintained, results in extensive liver cell necrosis (6, 11) Local deprivation of these amino acids may play an important part in ischæmic necrosis It is not improbable that the striking effects of the thio-amino-acids in reducing the susceptibility of protein depleted dogs to liver injury by chloroform (2, 20), may result from the increased blood level of these substances compensating for the intralobular deprivation consequent upon ischæmia

Constriction of the intralobular sinusoids after exposure to carbon tetrachloride could arise in one of two ways , by an action on the sinusoids themselves, or by their compression from without by the swollen parenchyma The sinusoids might be affected either by a direct constricting effect of carbon tetrachloride on their walls, or by a nervous reflex The latter explanation was suggested by Loeffler and Nordmann (16) and there is evidence that the general intrahepatic circulation is under some degree of nervous control (5, 9), although none that carbon tetrachloride acts in this way Such an action on the sinusoids, however mediated, might well explain the transient constriction following momentary exposure to carbon tetrachloride (24) and in that case the possibility can be entertained that the swelling of the parenchymal cells, after a longer exposure, is the result of a more prolonged constriction But there are other conditions with centrilobular degeneration in which such an explanation does not seem tenable A similar necrosis may occur when the liver is grossly infiltrated with fat (10) , it also occurs when the organ is loaded with inert substances like the polyvinyl alcohols (12) In the former narrowing of the sinusoids has been demonstrated (16, 7) In both these cases the swelling of the parenchyma is evidently due to the accumulation within the cells of a substance which there is no reason to believe has any pharmacological action upon the sinusoids themselves If swelling from such substances can lead to centrilobular degeneration it is improbable that the more pronounced swelling of the parenchyma due to carbon tetrachloride would be without effect

It appears, therefore, that the restriction of the intralobular circulation which becomes evident after exposure to carbon tetrachloride might well be due to swelling of the parenchyma Circulatory restriction of the degree observed could account, not only for the development of the subsequent necroses, but particularly for their characteristic localisation in the centrilobular zones Such an explanation would resolve the problem as to why necrosis develops in the central zones of the lobule, where the concentration of poison is low, while the parenchyma survives unscathed at the periphery where the concentration is high It also indicates how anoxia or malnutrition increase susceptibility of the liver to poisons and how administration of oxygen, or appropriate feeding, restores its normal resistance to such poisons

SUMMARY

1 The effect of carbon tetrachloride upon the intrahepatic circulation has been studied in rats by *in vivo* injection of Mandarin black into the spleen

2 Great restriction of the intra lobular circulation occurs soon after the administration of carbon tetrachloride and persists for many hours

3 It is suggested that this circulatory impairment accounts for the characteristic localisation of carbon tetrachloride necrosis in the centrilobular zones, and for its enhancement by anoxia and malnutrition

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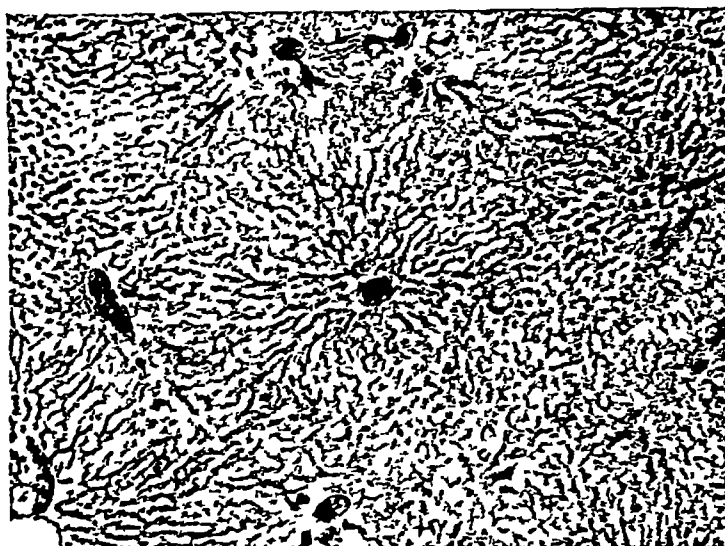
Fig 1 (/ 65) Liver from control rat showing the extensive penetration of the sinusoids by *in vivo* injection of Mandarin black
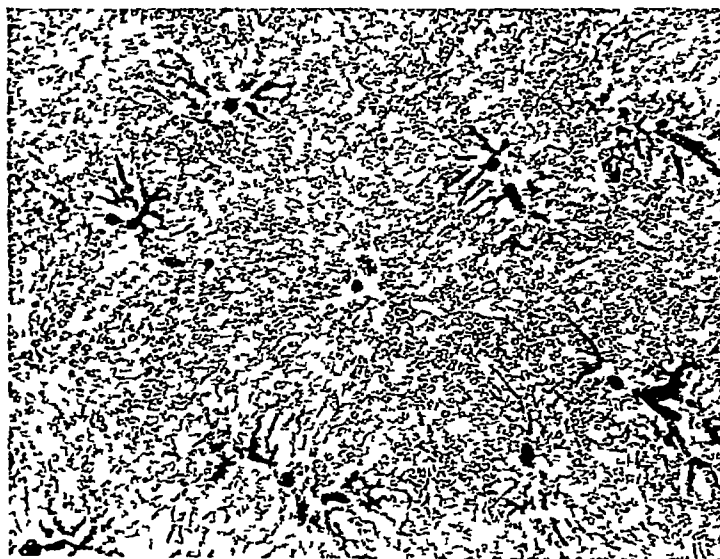
Fig 2 (/ 65) Liver from rat two hours after subcutaneous injection of carbon tetrachloride The sinusoids are extremely narrowed and the injection mass scarcely penetrates beyond the periportal zone
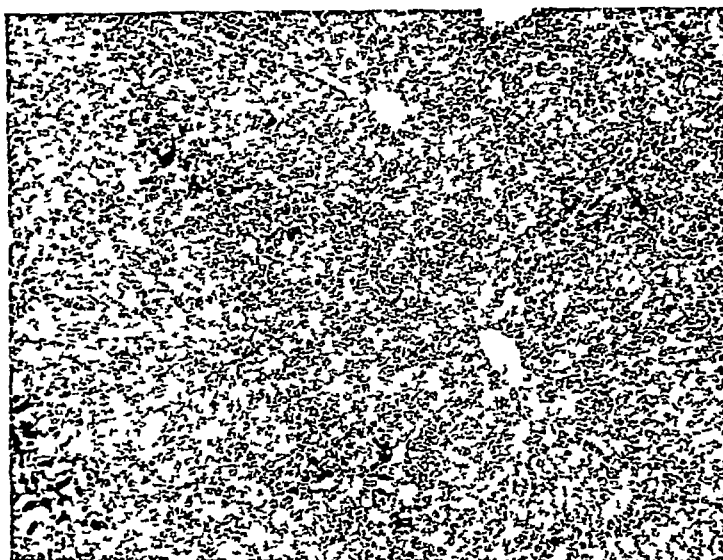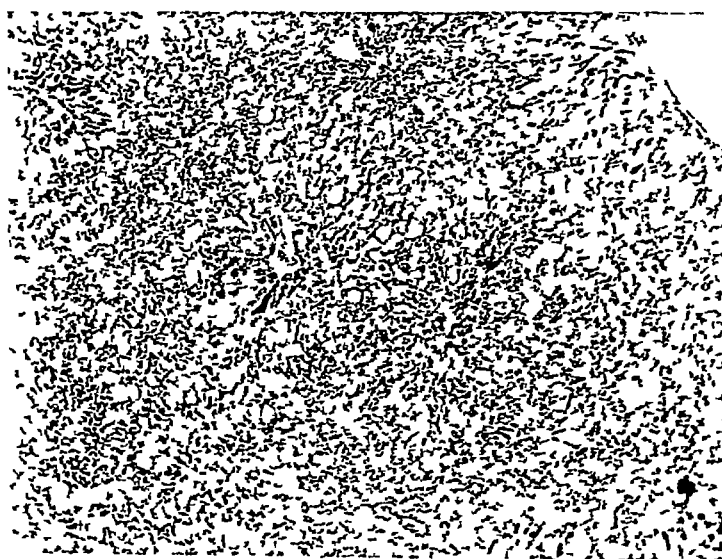
Fig 3   (✕ 65)   Liver from rat four hours after subcutaneous injection of carbon tetrachloride The sinusoidal spaces are almost completely obliterated except for a narrow zone around each portal tract

Fig 4   (✕ 65)   Liver from rat twenty four hours after subcutaneous injection of carbon tetrachloride The majority of the sinusoidal spaces are still obliterated Extensive centrilobular necrosis is conspicuous

LOW BLOOD PRESSURE IN DIABETIC COMA

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IT has long been recognised that in the later phases of diabetic coma the blood pressure may fall to low levels, and that this change is often irreversible, death following within a few hours   Thus Collen (2), in a series of 315 cases of diabetic coma, found a mortality of 86 per cent in patients who had a systolic blood pressure below 60 mm Hg   Efforts to raise the arterial pressure by drugs have been on the whole unsuccessful, while large intravenous infusions, given in the belief that the falling blood pressure was due to a diminished blood volume and a decreasing cardiac output, have frequently resulted in pulmonary œdema

Analysis of low blood pressure states depends on the principle that mean blood pressure varies directly with the product   cardiac output × total peripheral resistance   If the mean arterial pressure is low, then either the cardiac output or the total peripheral resistance, or both, must be diminished This principle has already been applied to the analysis of other low blood pressure states   In the "faint" or vasovagal reaction occurring during or after acute bleedings, the low blood pressure is due to diminished peripheral resistance from muscle vasodilatation (1) while in later phases after hæmorrhage both peripheral vasodilatation and lowered cardiac output may play a part (4)

The same principle has been applied to the analysis of cases of severe diabetic coma and the results are presented in this paper

Methods

The methods used have been described elsewhere (6)   Cardiac catheterisation was performed to measure right auricular pressure and to obtain samples of mixed venous blood   Arterial oxygen saturation was obtained from analysis of arterial blood samples   Satisfactory measurements

TABLE I

DIABETIC COMA

	INITIAL DATA			CIRCULATORY DATA						BIOCHEMICAL DATA			
Case	Age Sex	Ref No	Duration of coma on admission	B P mm Hg	Pulse	R.A.P (cm saline from sternal angle)	Arterio-venous O₂ diff (c c /lit)	Cardiac output (lit/min)	Total peripheral resistance (% normal)	Blood sugar mg %	Alkali reserve c c %	Blood urea mg %	Plasma chlorides (as NaCl) mg %
1	42 F	7 169	Precoma 1 day Coma 8 hrs	88/48	92	−4	31.0	8.05	42.5	1030	10.6	133	607
2	71 M	7 25	? about 12 hrs	68/35	96	+1.5	34.8	5.85	44.5	1000	14.7	175	604
3	48 F	5 6	Precoma 20 hrs Coma 12 hrs	30/	84	−3.0	55.1	4.5	33.5	652	12.0	150	691
4	51 F	5 5	Coma 18 hrs	55/0	104	−2.0	41.5	6.0	46.0	678	12.0	102	657
5	41 F	6 50	Precoma 18 hrs Coma 5 hrs	110/70	80	+1.0 V.P.	12.1	—	—	495	10.9	65	630

TABLE I (continued)

Case	Total glucose	Total insulin	Total intravenous fluids	Result	Remarks
1	150 g i v	950 i v 800 i m	2,880 c c	Died 10 hrs after admission	Initial data obtained after 350 u insulin and 500 c c 10% glucose saline + 250 c c isotonic sod bicarbo nate. Acidosis corrected without affecting circulatory state
2	100 g i v 25 g by mouth	2,000 i v	1,090 c c	Died 12 hrs after treatment commenced	Urine acetone free on admission and subsequently Acidosis corrected after 6 hrs without affecting circulatory state
3	324 g i v	2,140 i v	3,210 c c	Died 9 hrs after admission	Acidosis corrected terminally (alkali reserve 52 8 c c %)
4	105 g i v	210 i v 50 i m	1,300 c c	Died 5½ hrs after admission	Initial data obtained after 70 g glucose + 110 i v } units 50 i m } insulin
5	170 g i v	700 i v 50 i m	1,700 c c	Died ½ hrs after admission	Catheter in R innominate vein, cardiac output not calculable B P fell to 68/10 ½ hr before death

of oxygen consumption were made in Case 2, (204 c c /min) and in Case 10 29 (not included in the present series) Case 10 29 had an oxygen consumption of 272 c c /min and other data on this case were as follows systolic blood pressure 76 mm Hg , heart rate 88 per minute, right auricular pressure 9 cm below sternal angle level, arteriovenous oxygen difference 42 c c /litre, and cardiac output 6 5 litres/min The blood sugar in this case was 408 mg /100 c c and the CO_2 combining power 9 vols/100 c c In the remaining cases, records of oxygen consumption were not satisfactory owing to the difficulty of preventing leaks in comatose patients An average figure

TABLE II

Case	Age Sex	Ref No	Circulatory Data						Remarks
			B P mm.Hg	Pulse	R A P cm of saline above S.A	Arterio venous O_2 diff (c c /li)	Cardiac output (li/min)	Total periph resist (% of normal)	
6	40 F	5 1	80/60	—	—	—	—	—	On admission Blood sugar = 608 mg % Alkali reserve = 15 7 c c % Blood urea = 107 mg % Plasma chlorides = / 580 mg %
			125/50	100	—5	38 0	6 6	66 5	10 hrs later after 1000 c c 10% glucose saline and 140 units insulin intravenously Urine sugar and acetone free
			118/75	88	—7	43 0	5 1	95	1 month later

of 250 c c /min has therefore been assumed in these cases If the low blood pressure is to be explained by a reduction in cardiac output, then the oxygen consumption would have to be about half this average resting figure Our own measurements, in two cases, and those of Dubois (3) do not indicate that oxygen consumption is reduced in diabetic coma

Mean blood pressure was not measured directly but was assumed to be systolic B P + diastolic B P
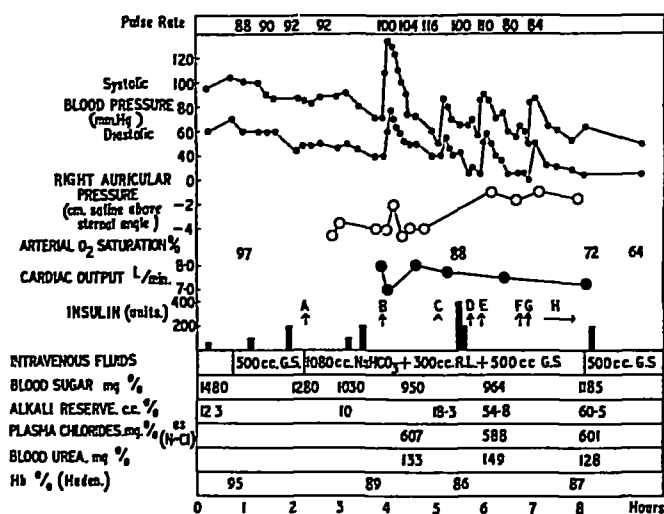
Fig 1   Treatment and progress record of Case No 1
          A = Digoxin 1 5 mg intravenously
          B = Pitressin 10 units intravenously
          C = Pitressin 10 units i v
          D = 100 c c saturated soda bicarbonate intravenously
          E = Pitressin 10 units i v
          F = Coramine 4 c c i.v
          G = Pitressin 10 units i v
          H = Pitressin 30 units added to drip
   In this and in subsequent figures GS = 10% glucose saline
                                      RL = Ringer lactate solution.
                          NaHCO₃ c c = isotonic solution.
                          NaHCO₃ g = soda bicarbonate dissolved in drip fluid.
   Pitressin 10 units intravenously produced an initial temporary rise in blood pressure, but
subsequent injections had little effect   Arterial oxygen saturation showed a steady fall
throughout to a low terminal figure
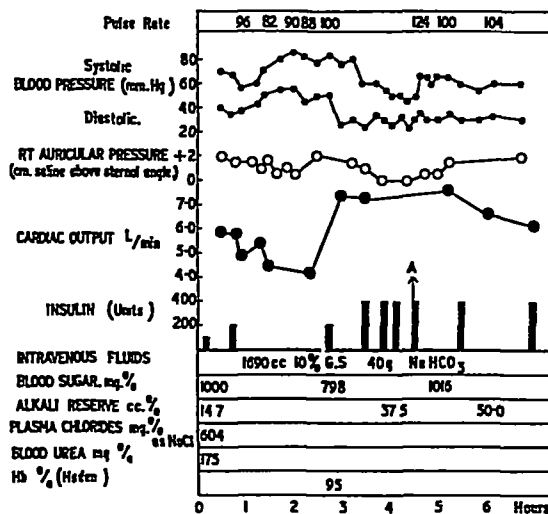
Fig 2   Treatment and progress record of Case No 2
          A = Methedrine 15 mg intravenously
   The increase in cardiac output at the 3rd hour was associated with an increase in heart rate,
and a further fall in blood pressure indicated increasing vasodilatation.

Total peripheral resistance was calculated from this formula —

$$TPR = \frac{Mean\ B\ P}{cardiac\ output}$$

and expressed as a percentage of the normal, assuming a normal mean arterial pressure of 100 mm Hg and cardiac output of 5 litres/min All patients were supine at the time of the observations Intravenous infusions in most cases were given through the catheter this procedure was found convenient, since venous spasm, early venous thrombosis and further disturbance of the patient were avoided Insulin was given both intravenously and intramuscularly the doses used are reported in Table I Blood sugar estimations were performed on capillary blood by Harding's method, and blood urea, CO_2 combining power and plasma chlorides on mixed venous blood by the methods of Nessler (Jackbean), van Slyke, and King and Haslewood respectively

Results

Initial circulatory and biochemical data in 5 cases of severe diabetic coma are shown in Table I, and details of the subsequent treatment in Figs 1 to 5 A severe degree of acidosis existed in every case Cardiac output was above the normal average (5 3 l /min) in 4 cases and normal in 1 The right auricular pressure was within normal limits or slightly raised in every case Three of the cases showed an increased heart rate Total peripheral resistance was always markedly reduced, and there was evidence that vasodilatation might persist in spite of successful treatment a sixth case with a low blood pressure on admission was studied 14 hours after the commencement of treatment when the urine was sugar and acetone free (Table II) Cardiac output was still high and peripheral resistance low at this time A further observation one month later showed a normal circulatory state

Effects of treatment

(a) *Glucose, insulin and alkalies* In Cases 1 to 5, treatment was unsuccessful and the patients died Though the total fluid given intravenously (1300—3240 c c) did not seem excessive in view of the dehydration, a progressive fall in arterial oxygen saturation was noticed in the terminal stages, and pulmonary œdema was present at autopsy in the four cases so examined In spite of the large doses of insulin used, the blood sugar level was little affected The correction of the blood acidosis by alkalies added to the glucose saline infusions in three cases failed to alter the circulatory state

(b) *Vasoconstrictor drugs* Attempts to raise the arterial pressure by Methedrine (d-N-methyl amphetamine hydrochloride) resulted only in a transitory rise , a second injection was given in one case without effect Intravenous pitressin, 10 units, raised the blood pressure for a quarter of an hour in one case, but three further injections produced only small
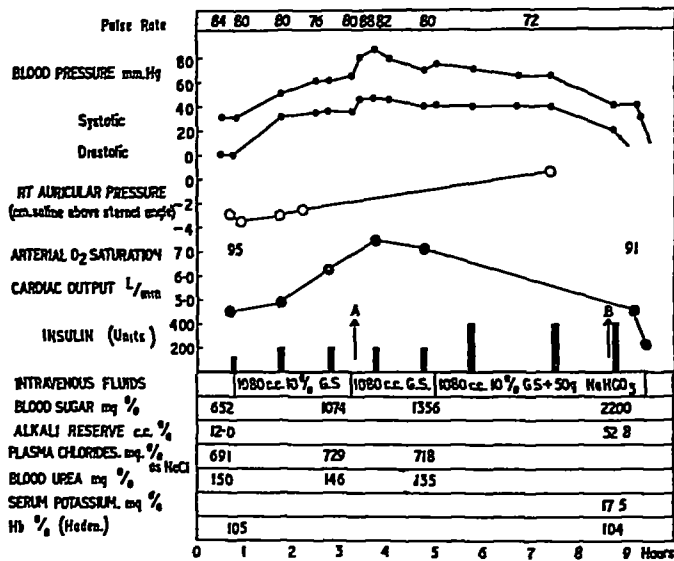
Fig 3   Treatment and progress record of Case No 3
          A = Methedrine 30 mg intramuscularly
          B = Methedrine 60 mg intramuscularly
     There was a small rise of blood pressure after an injection of methedrine, but a later larger
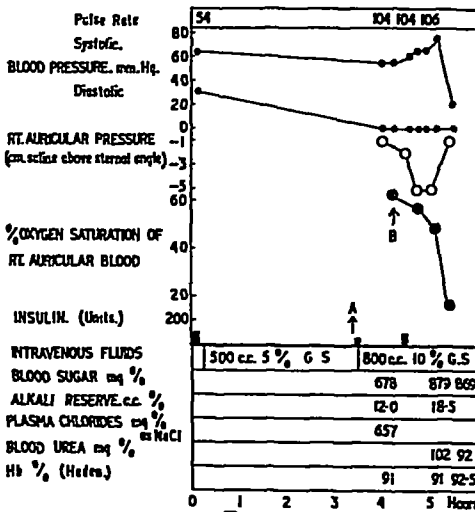injection was without effect   Arterial oxygen saturation showed only a small terminal fall

Fig 4   Treatment and progress record of Case No 4
          A = Digoxin 1 5 mg intravenously
          At B arteriovenous oxygen difference = 41 5 c c /litre
                         Cardiac output = 6 0 1 /min
          Arterial oxygen saturation = 83%
     Only one arterial sample was obtained   Percentage oxygen saturation of right heart blood
is therefore plotted and the terminal fall in this saturation was probably in part due to a
diminishing arterial oxygen saturation
Fig 5   Treatment and progress record of Case No 5
     The catheter in this case was in the right innominate vein   Percentage oxygen saturation
of blood taken from this situation is therefore plotted   A very small arteriovenous oxygen
difference indicated a rapid flow
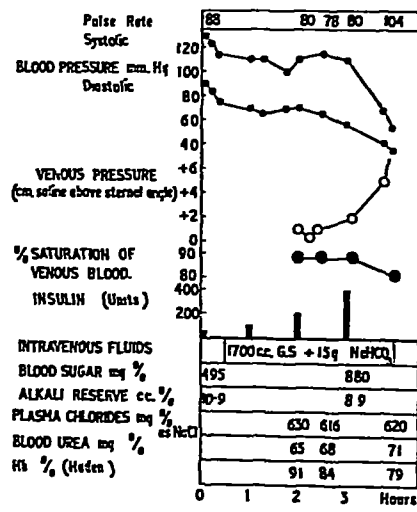
changes, and no effect was noticed when the drug was added to the intravenous drip Intravenous digoxin, which often produces a rise in arterial pressure, and coramine were both without effect on the blood pressure

Discussion

Right auricular pressure may be considered as depending on blood volume and on venous tone   If there is any diminution of blood volume in the later phases of diabetic coma it appears to be fully compensated by vasoconstriction, right auricular pressure being normal or slightly raised in every case   Since cardiac output is normal or increased, and there is no central cause for the low blood pressure state, little benefit can be expected from intravenous infusions which raise still further the right auricular pressure   Such infusions may be dangerous and lead to pulmonary oedema and a progressively falling arterial oxygen saturation   It might be rational, therefore, to restrict intravenous fluids to a minimum

The low blood pressure in diabetic coma is due to a decreased total peripheral resistance, which is below 50 per cent of the normal value   The site of the vasodilatation has not been determined   The cool pale skin suggests that skin blood flow is diminished   Decreased arteriovenous oxygen differences of femoral vein samples found by Schecter, Wiesel and Cohn (7), and interpreted by them as evidence of decreased oxygen utilisation, may also mean that vasodilatation exists in muscle vessels   The cause of the peripheral vasodilatation is obscure, but it may possibly be related to tissue acidosis, since an apparently similar circulatory state exists in aspirin poisoning, uraemia, and the terminal phases of heart failure from emphysema (5)   Restoration of the CO_2 combining power of the blood to normal, however, may fail to check the falling blood pressure   and, after successful treatment, vasodilatation may persist for many hours (Table II)   Attempts to combat the peripheral vasodilatation by constrictor drugs have so far failed   The vessels may show a transient initial constriction, but then appear to become insensitive to further injection of the drugs   The problem of how to increase the peripheral resistance and raise the blood pressure therefore remains

SUMMARY

Cases of severe diabetic coma in the low blood pressure phase have been investigated and the results analysed on the principle —mean blood pressure = cardiac output × total peripheral resistance

The low blood pressure is due to a greatly decreased total peripheral resistance, since cardiac output is not reduced

Restoration of CO_2 combining power to normal levels may have little effect on the peripheral vasodilatation

The vasoconstrictor drugs pitressin, digitalis and d-N-methyl amphetamine hydrochloride (Methedrine) fail to raise the low arterial pressure, or show only a small transient effect

Large intravenous infusions may cause pulmonary œdema and a falling arterial oxygen saturation

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THE ESTIMATION OF THE EXTRACELLULAR FLUID VOLUME BY THE THIOCYANATE METHOD IN CHILDREN AND ADULTS

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A SIMPLE method for measuring the extracellular fluid (ECF) volume would be useful in many clinical problems involving changes in hydration Previous investigators have reported reliable results using a method where sodium thiocyanate (NaSCN) is injected intravenously and the volume of body water into which it diffuses is estimated It was first shown by Crandall and Anderson (4) that if NaSCN is injected intravenously into man or animals the serum NaSCN level falls rapidly for a time, later falling more slowly On the assumption that this was due to the thiocyanate becoming distributed in some fluid compartment of the body, they estimated the volume of this " thiocyanate available fluid " (TAF) This and subsequent studies (1, 2, 6, 10, 11, 12, 13, 21) in animals and man, some of them (10, 21) simultaneously estimating TAF and sodium space by radioactive sodium, sought to define the physiological compartment which was represented by the TAF These showed that the latter was closely related to, though not identical with the ECF, and that in dehydration the loss of TAF was approximately the same as the loss of ECF The method thus seemed to offer a means for determining changes in ECF volume in states of altered hydration, and has been used for this purpose in adults (8, 10, 16, 18, 20) and in a few cases in children (14)

The present work was undertaken to assess the value of the method in investigating problems of dehydration, particularly in infants, the evaluation has involved —

1 Definition of the optimum conditions for the determination of the TAF

2 Repeated determinations of the TAF in order to discover within what limits this varies in the same subject

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3 Determination of the TAF in normal subjects of different ages in order to discover if the TAF constitutes a constant fraction of the body dimensions

1 Optimum conditions for the determination of " thiocyanate available fluid "

Methods Sodium thiocyanate (British Drug Houses, technical quality) was dissolved in distilled water, filtered, sterilized by boiling and put up in sterile sealed 1 ounce bottles   Solutions of approximately 5% and 2% NaSCN were made and subsequently standardized by titration against AgNO$_3$ of known strength, using ferric alum as indicator   Solutions were tested for sterility   Toxicity is of no importance when small single doses are used (5, 19), but the presence of toxic impurities was tested for, by giving a rabbit an intravenous dose of NaSCN equivalent to 4 times the maximum dosage employed in our human studies   the animal remained unaffected   Kept in a refrigerator the solutions remained of constant strength for at least 3 months

Dosage of NaSCN to give any required serum level was calculated by assuming a TAF volume of 30% of body weight   4 – 25 ml of the solution were injected intravenously from a syringe calibrated to deliver known volumes   This injection was sometimes made through a needle which had been inserted into a vein for previous blood sampling , it was shown that no significant error resulted   The vein used for injection was not subsequently used for sampling   Before the injection a blood sample was taken to serve as a blank in subsequent estimations, since normal sera contain small amounts of substances which give a colour reaction similar to thiocyanate (6, 7)

Ferric ions react with thiocyanate to form a coloured complex and this provides a method for estimating small amounts of thiocyanate   The reagents used by Crandall and Anderson (4) have been employed by all subsequent workers in this field who have tacitly accepted the reliability of the technique   Recently however, Bowler (3) has found that their method gives results about 7 5% too low when applied to serum   He has defined the optimum conditions for the reaction and claims that reliable results are thus obtained up to serum concentrations of 6 mg % of NaSCN   We have used Bowler's method and estimated the colour intensity using a Hilger-Spekker absorptiometer with Calorex heat screens (H 503) and Whatten blue filters (No 50, maximum transmission at 4550 Å), the solutions being contained in cells of 1 cm thickness   3 ml blood provides sufficient plasma or serum for the determination

Different amounts of a 0 005% NaSCN solution were added to 1 25 ml 20% trichloracetic acid and this was made up to 5 ml   The amounts of NaSCN were such that if dissolved in 1 ml the resulting concentration would range up to 25 mg % w/v   It is with this meaning that " concentration of

NaSCN " is used in giving the results, since in applying the method to serum 1 ml of serum was the amount used 5 ml 16% Fe (NO$_3$)$_3$ 9H$_2$O in 1N HNO$_3$ were added to the above and the colour developed compared with that of a blank of 1 25 ml 20% trichloracetic acid with 3 75 ml water similarly treated Results are shown in Table I

TABLE I

Colour intensity produced by ferric nitrate with aqueous solutions of thiocyanate

NaSCN mg %	Colour intensity (extinction coefficient)	Number of estimations	Maximum deviation from mean
5	0 119	7	2 5%
10	0 232	5	2 5%
15	0 343	5	1 0%
20	0 451	4	1 6%
25	0 561	2	0%

These results show a direct proportionality between NaSCN concentrations and colour intensity (Beer's law) up to NaSCN concentrations of 15 mg %, each 1 mg % being equivalent to a reading of 0 0231 At concentrations of 20 mg % or over there is a slight falling off of colour intensity

The method was applied to serum as follows Different amounts of 0 005% NaSCN were made up with water to 6 5 ml , 1 ml serum and 2 5 ml 20% trichloracetic acid were added, the mixture well shaken, left to stand 10 minutes and filtered through a Whatman No 42 paper 5 ml of the filtrate was treated with 5 ml of the ferric nitrate reagent and the colour intensity compared with that of a blank prepared similarly omitting the NaSCN Results are shown in Table II where the concentration of NaSCN has the same sense as before

TABLE II

Colour intensity produced by ferric nitrate with thiocyanate in serum

NaSCN mg %	Colour intensity (extinction coefficient), mean of 4 estimations	Maximum deviation from mean
5	0 110	1 4%
10	0 221	0 4%
15	0 329	0 6%
20	0 430	0 7%

Inspection of these figures shows that Beer's law is again obeyed up to NaSCN concentrations of 15 mg %, each 1 mg % being equivalent to a reading of 0 0220　Comparison with the results in aqueous solution shows a loss of 4 7%, which is constant and true for all concentrations up to 20 mg %　This is presumably due to some thiocyanate being carried down with the precipitated proteins

Using a calibration curve based on these results with serum, the reliability of the method was checked by a series of 9 recoveries using serum concentration 5 – 19 mg % NaSCN　The mean recovery was 99 8% and the maximum error 2 1%　Recovery of thiocyanate from heparinised plasma (0 02 ml or 100 I U heparin to 3 – 20 ml blood) showed similar results of 4 specimens of plasma containing 5 – 20 mg % of NaSCN, the mean recovery was 99 2% and the maximum error 2 5%　Thus heparinised plasma can be used in place of serum and either has been used according to convenience
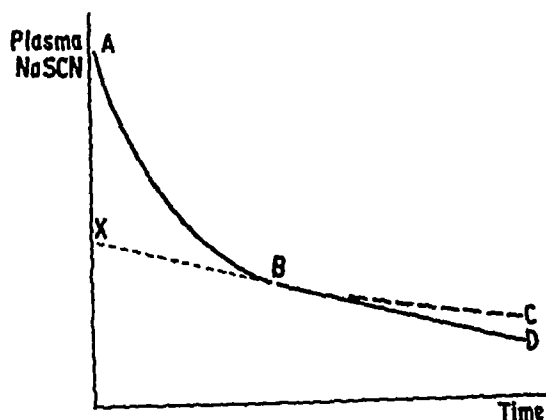

Fig 1　Typical form of disappearance curve of NaSCN from plasma (see text)

Thiocyanate diffusion equilibrium　Following an intravenous injection of NaSCN the curve obtained by plotting plasma NaSCN levels against time (Fig 1) consists of two components, the first (ABC) of exponential form, reflecting the rapid fall due to diffusion of NaSCN from the plasma into the interstitial fluid , the second (BD) showing a slow fall due to disappearance of NaSCN from the TAF　The slope of the second curve is so small that it may in practice be regarded as of linear form　The junction of these two components, point B, represents the point at which diffusion equilibrium is considered for practical purposes as complete, and this point requires to be determined in order that the volume of TAF may be measured　The discordance of the results of others (4, 10, 18, 19) as to the time in which diffusion equilibrium is attained required that this matter be studied afresh

In 8 preliminary experiments upon 2 adults and 3 infants it was found that the disappearance rate of NaSCN from plasma after an intravenous

injection yielding plasma levels of 4 5 – 12 mg % usually became slow only towards the end of the second hour   In 7 experiments the disappearance curve was followed from 2 hours up to 6 – 30 hours   During this period the hourly fall of plasma NaSCN varied from 1% to 4% of the plasma level, averaging 1 4%   Since diffusion equilibrium was usually not attained before 2 hours, subsequent experiments aimed at defining the rate of disappearance after this time

Plasma NaSCN levels of 5 – 9 mg %   17 experiments were performed, 14 on normal subjects including 2 adults, 7 children 1 – 12 years and 3 infants 4 – 6 months old , in addition 3 moderately dehydrated infants 6 – 11 months old were investigated   The first sample of blood was taken 2 hours after injection of NaSCN and a second sample 3 to 7 hours after injection The percentage of the 2 hour plasma NaSCN level which was subsequently lost per hour was —

in	6 experiments	0 – 3% loss,
„	5 „	3 – 6 5 % loss,
„	5 „	6 5 – 10% loss,
„	1 „	16% loss

In view of the erratic results at these levels and of the high disappearance rate still continuing in some cases after 2 hours, lower plasma levels were subsequently employed

Plasma NaSCN levels of 3 – 5 mg %   33 experiments were made on 21 normal subjects including 2 adults, 15 children and 4 infants 3 – 11 months of age

The percentage of the 2 hour plasma NaSCN level which was lost or gained during the subsequent 1 hour was —

in	14 experiments	0 – 3% loss,
„	8 „	3 – 6 5% loss,
„	5 „	6 5 – 20% loss,
„	1 „	0 – 3% gain,
„	2 „	3 – 6 5% gain,
„	3 „	6 5 – 15% gain

Results are thus less erratic at these lower levels for in 25 of the 33 (75%) experiments, the level at 3 hours did not differ from that at 2 hours by more than ± 6 5%   Age did not influence the degree of coincidence between the 2 and 3 hour levels

In the light of these findings the 2 hour value has been accepted as sufficiently representing the diffusion equilibrium level only if the level during the subsequent hour has not altered by more than ± 6 5%, this figure having been chosen arbitrarily   25 of the 33 experiments at low plasma levels and 11 out of 17 at higher levels are thus acceptable for determination of the TAF, a total of 36 experiments

In view of the variable rate of disappearance of NaSCN between 2 and 3 hours, both as between different subjects and, as we have also observed, between different experiments on the same subject, extrapolation (16) of the disappearance curve back to zero time (point X, Fig 1) was rejected as a method for determining the equilibrium concentration, and the 2 hour value was considered preferable for this purpose

In order then to calculate the TAF, the amount of NaSCN injected was divided by the equilibrium NaSCN plasma concentration   Attempts such as have been made by Lavietes (12, 15) to elaborate the calculation in order that the TAF shall bear a closer relationship to the ECF may introduce larger errors (8, 9, 19) than those they seek to correct, besides requiring an estimate of the blood volume

Urinary excretion of thiocyanate   A knowledge of the urinary excretion of thiocyanate would be helpful in elucidating the factors concerned in its disappearance from the ECF

It was found that the colour developing after the addition of ferric nitrate reagent to urine containing known amounts of NaSCN was more than double that which could be accounted for by the NaSCN present, due probably to the coloured compounds formed by the reaction of urinary chromogens with ferric ions   Such gross interference precludes the use of a colorimetric method employing a ferric salt   Previous attempts to do so will be discussed later

2   The reliability of " thiocyanate available fluid " estimations

In order to check the reliability of the method repeated determinations of the TAF were made in a series of normals   Similar NaSCN plasma levels were generally employed in a repeated experiment on a subject   this was achieved by injecting a proportionately smaller amount of NaSCN if the time interval between the two injections was short (less than 6 days)   As a blank for the second estimation a blood specimen collected immediately before the second injection was used, hence the increase in NaSCN concentration, not its absolute amount, was measured

Repeated estimations were made on more than 20 subjects but after discarding those in which diffusion equilibrium as defined above was not attained at 2 hours there remained 9 cases, 7 children upon each of whom 2 estimations were made and 2 adults upon each of whom 3 estimations were made

Estimations were always made under comparable conditions as regards time of day and relation to meals   Results are given in Table III where the last column shows the difference between the highest and lowest values of TAF/body weight, allowance thus being made for any change in TAF due to change in body weight between the estimations

TABLE III

Variability of successive determinations of " thiocyanate available fluid " (TAF) in normal subjects

Subject	No of Experiment	Age (years)	Interval between successive estimations (days)	Weight (kg)	TAF (litres)	TAF (% of body weight)	Variation in TAF (% of mean value)
W.F	(1)	7		19 11	6 37	33 3	
,,	(2)	,,	5	19 11	6 18	32 3	3 5
D G	(1)	36		70 00	18 40	26 3	
,,	(2)	,,	130	72 20	18 90	26 1	3 9
,,	(3)	,,	6	72 20	18 28	25 3	
L T	(1)	3		19 07	5 07	26 6	
,,	(2)	,,	5	19 07	4 86	25 5	4 2
J U	(1)	11		28 50	7 59	26 6	
,,	(2)	,,	5	28 50	8 00	28 0	5 1
D C	(1)	1½		12 08	3 26	26 9	
,,	(2)	,,	5	11 80	2 99	25 3	6 1
J.B	(1)	12		31 50	9 07	28 8	
,,	(2)	,,	6	31 50	8 51	27 0	6 4
A B	(1)	1$\frac{2}{12}$		9 02	3 38	37 4	
,,	(2)	,,	44	9 36	3 27	34 9	6 9
S.D	(1)	29		77 30	17 01	22 0	
,,	(2)	,,	129	77 30	15 74	20 4	10
,,	(3)	,	6	77 30	15 40	19 9	
P H	(1)	6		19 75	4 86	24 6	
,,	(2)	,	5	19 75	5 69	28 8	15

3 *The relation of the " thiocyanate available fluid " to body weight and surface area at different ages*

In addition to repeated estimations made on the 9 cases given in Table III, single estimations made on a further 16 infants and children (13 normal, 3 moderately dehydrated) fulfilled the criteria of validity as defined

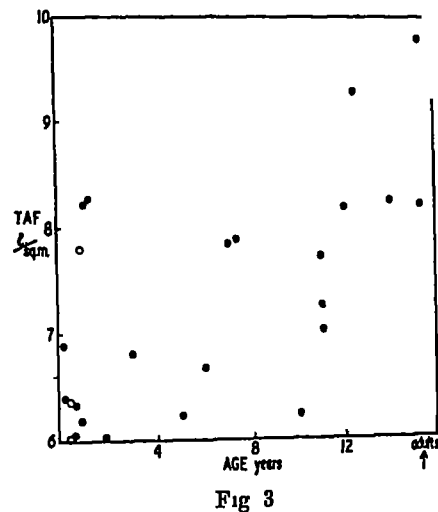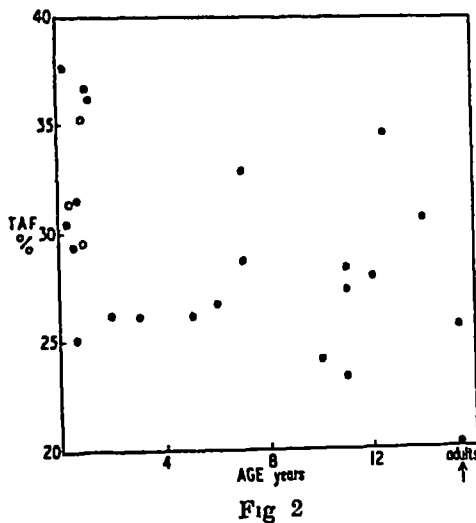The clinical details of the 3 dehydrated infants were

Case 1 Age 6 months, male Weight, 6 40 kg Gastroenteritis of 7 days duration, with a weight loss during this period of 600 gm A

moderate degree of dehydration was shown by the depressed fontanelle, dry tongue and loss of skin turgor

Case 2 Age 6 months, male Weight, 7 31 kg Tuberculous meningitis of 11 days duration Moderate degree of dehydration as shown by dryness of mouth and early impairment of skin turgor

Case 3 Age 11 months, male Weight, 8 40 kg Tuberculous meningitis of 18 days duration Signs of moderate dehydration had become evident only during the latter 4 days, the weight loss over this latter period being 300 gm

The results of these 25 estimations of TAF (taking the mean value of repeated estimations) expressed as a fraction of body weight and of surface area and plotted against age are shown in Figs 2 and 3

Fig 2

Fig 3

Figs 2 and 3 " Thiocyanate available fluid "/body weight (Fig 2) and " thiocyanate available fluid "/surface area (Fig 3), in 22 normal subjects (dots) and 3 dehydrated infants (circles)

The TAF as a proportion of body weight varied in normals from 23% to 38% and as a proportion of surface area from 5 9 to 9 8 litres per square metre the 3 dehydrated cases fell within these normal limits

4 *Discussion of results*

Thiocyanate diffusion equilibrium The practical problem has been to determine the plasma NaSCN level close to the point (point B, Fig 1) where diffusion equilibrium has been attained, while employing the minimum number of blood samplings Since the work was intended to be applicable to infants this latter was of special importance Further, if the method is to be applicable to clinical problems of dehydration samplings cannot be continued over a long period since changes of hydration might take place within this time and since initiation of treatment cannot be long delayed

The time required for diffusion to be complete in man has been variously found by previous workers to be 25-35 minutes (19), 2 hours (4) and 3 hours (10), while most have stressed that in some subjects the time required may be much longer than in the majority   Our findings have shown that at least 2 hours is usually required before there is appreciable slowing of the disappearance rate to the 1 − 4% hourly loss of plasma NaSCN which obtains after diffusion equilibrium is complete   Although in some cases, equilibrium may still be incomplete at 2 hours, later samples include increasing error from the continuous slow loss of NaSCN from the TAF   The 2 hour level was thus chosen as most likely to correspond to diffusion equilibrium, and was accepted as doing so if the level 1 hour later did not differ from it by more than ± 6 5%   This condition obtained in 75% of the experiments using low (< 5 mg %) plasma NaSCN levels

The remaining 25% of these experiments in which the 2 hour and 3 hour levels differed by more than 6 5% included 5 experiments where the 3 hour level was lower than the 2 hour by more than 6 5%, probably due to diffusion equilibrium having been incompletely attained at 2 hours   In 3 experiments the 3 hour level exceeded the 2 hour level by more than 6 5% , the reason for this is unknown, previous workers (4, 10) having also found that the plasma level may rise somewhat between 2 and 4 hours

Reliability of " thiocyanate available fluid " determinations   The agreement between repeated determinations of TAF (Table III) was satisfactory in 7 out of 9 subjects where values differed by less than 7%   In the remaining two subjects, differences amounted to 10% − 15%   In one of these subjects (S D) three determinations were made within a period of 4 months, the weight remaining constant during this time   The second and third determinations, which were made within 6 days, differed by only 2 5%, while the first determination made 4 months earlier differed from the later determinations by 10%   This raises the possibility (10) that under different climatic or other conditions the TAF may vary   The only other duplicate estimations on normal men were made (10) on four adults , the differences between repeated estimations ranged from 2 3 to 4 1%   Repeated estimations on normal dogs showed differences as high as 21% (1, 6, 9)

Relation of " thiocyanate available fluid " to age   Figs 2 and 3 display the wide variation in the normal TAF whether expressed as a proportion of body weight or of surface area   For example the TAF (either in terms of weight or surface) of two individuals of the same age and similar nutrition and physique may differ by as much as 50%   Previous workers (12, 14, 17, 19) have found a similar variation   There is some tendency for the TAF to decrease with age when expressed as a proportion of body weight and to increase with age when expressed as a proportion of surface area

Estimation of thiocyanate in urine   The interference of urinary chromogens with the colorimetric estimation of urinary thiocyanate when

moderate degree of dehydration was shown by the depressed fontanello, dry tongue and loss of skin turgor

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Estimation of thiocyanate in urine   The interference of urinary chromogens with the colorimetric estimation of urinary thiocyanate when

using a ferric reagent has been described   Two methods have been proposed to overcome this difficulty   The first (12) employs as blank a urine of similar colour to the urine in which thiocyanate is to be measured   There is however no proof that urines of similar colour will develop the same colour intensity with ferric reagent   The second method (6) consists in diluting the urine so as to minimise the effect of the interfering substances   Since, however, the thiocyanate concentration is reduced at the same time there is no practical gain   The conclusions of these authors as to the urinary excretion of thiocyanate are therefore of doubtful validity

Conclusions   It has been shown that by taking blood samples 2 and 3 hours after the intravenous injection of thiocyanate an "equilibrium" plasma level and hence a valid determination of the TAF may be obtained only in 75% of subjects ranging from infancy to adult   Repeated estimations of the TAF on the same subject though often agreeing closely may vary by as much as 15%   In order to improve the reliability of the method it would be necessary to take a larger number of blood samples and to prolong the samplings beyond the 3 hour period

The 3 dehydrated infants fall within the normals   It is clear therefore that a single estimation of the TAF is unlikely to demonstrate or to measure dehydration

For these reasons the method has not proved readily applicable to clinical problems of dehydration

SUMMARY

1   A standard technique for the determination of the " thiocyanate available fluid " is described   In estimating thiocyanate in serum or plasma it is necessary to correct for the small amount of thiocyanate lost with the precipitation of the proteins   Heparinised plasma can be used in the same way as serum

2   Thiocyanate in urine cannot be estimated by the same method

3   Repeated determinations of the " thiocyanate available fluid " in 9 normals agreed well in many cases but in others differed by as much as 15%

4   The " thiocyanate available fluid " was determined in 22 normal subjects   In individuals of the same age and similar physique the " thiocyanate available fluid " may differ by 50%   The " thiocyanate available fluid " in 3 dehydrated infants was within normal limits

5   It is concluded that the determination of the " thiocyanate available fluid " is not a method which is readily applicable to the study of clinical problems of dehydration

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THE ACTION OF ADRENALINE, EPHEDRINE

AND *METHEDRINE ON THE CIRCULATION IN MAN

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Introduction

THERE are a few methods of measuring the effect of drugs on the circulation
in individual human tissues   Using the venous occlusion plethysmograph
Grant and Pearson (6) and Holling (8) found that small intravenous doses
of adrenaline increased the blood flow in human muscle and decreased the
flow in skin   Subcutaneous or intramuscular administration had the same
effects (1, 11)   The results of other methods of investigation agree with
these findings

In the experiments to be described these actions of adrenaline have
been confirmed and amplified by the venous occlusion plethysmograph,
and ephedrine and methedrine have been similarly studied   Both are well
known pressor agents whose vascular effects do not seem to have been
previously studied in detail

Methods

The subject lay semi-reclining on a couch in a warm (20°C) quiet room
Blood flow through the forearm and hand was measured by Barcroft and
Edholm's (3) modification of the method of Lewis and Grant (10) using a
bath temperature of 34°C for the forearm flow and 32°C for the hand   It
was found that the most satisfactory hand flows were obtained when the
hand was supported at shoulder level or higher   The veins of the hand are
very subject to congestion and if the limb is in a dependent position, or if
the rubber cuffs used to seal the hand into the plethysmograph are even
slightly tight fitting, the veins are so filled before the collecting cuff is
inflated that they can accommodate very little more blood, and poor

readings result   When the arm is elevated the hand veins tend to empty and so permit a more prolonged period of steady filling when the venous occlusion is applied   Blood flow results are expressed in c c blood per 100 c c forearm, or hand, per minute   The pulse was counted by palpation, and the blood pressure measured by auscultation

Measurements of blood flow, pulse and B P were made at 5 minute intervals until constant readings were obtained before any drug was injected Thereafter the experiment was continued until it became evident that the maximum effect had been produced, or until no further important changes took place

The injections were made into either the deltoid or quadriceps muscles The doses of the three substances investigated were —adrenaline, usually 1 0 c c of the 1/1000 B P solution (hereafter referred to as 1 0 mg), ephedrine, 60 mg and 90 mg of the natural l-hydrochloride, and methedrine 20 mg , in 1-2 c c saline

Most of the experiments were carried out on healthy medical students In the case of the sympathectomized forearms detailed post-operative tests were made on three only   These tests (finger temperature (9), hand blood flow (13) and skin resistance in response to indirect heating and nerve block) conducted by others in this department, have shown that in the three cases concerned sympathetic control was very slight or absent   In one instance ganglionectomy had been performed five years before, in the other two the operation of Smithwick (14,15) was employed, the times since operation being six weeks and one and a half years

RESULTS

Adrenaline   The dose used was 1 0 mg intramuscularly   The action was rapid, being conspicuous 1½ to 2 minutes, and maximum 5 minutes after completing the injection   Strong palpitation, tremor, epigastric discomfort, apprehension and sometimes headache or fullness of the head were invariably produced by this dose   Pallor was marked   After subcutaneous injection the effects began later and were less intense Following intramuscular injection the severe symptoms usually lasted no longer than 10 minutes, after which they were relatively mild, whereas the circulatory effects persisted for a considerable time   The tremor was the most persistent manifestation

The systolic pressure rise was much the same in all cases   Diastolic pressure fell to a varying degree, averaging 10 mm Hg (Fig 1)   The heart rate was only slightly and variably affected, averaging an increase of 10 beats per minute   Forearm blood flow also showed wide variation from case to case but an increase was always produced   Fig 1 shows that in seven cases the flow rose to an average of 2½ times its resting value
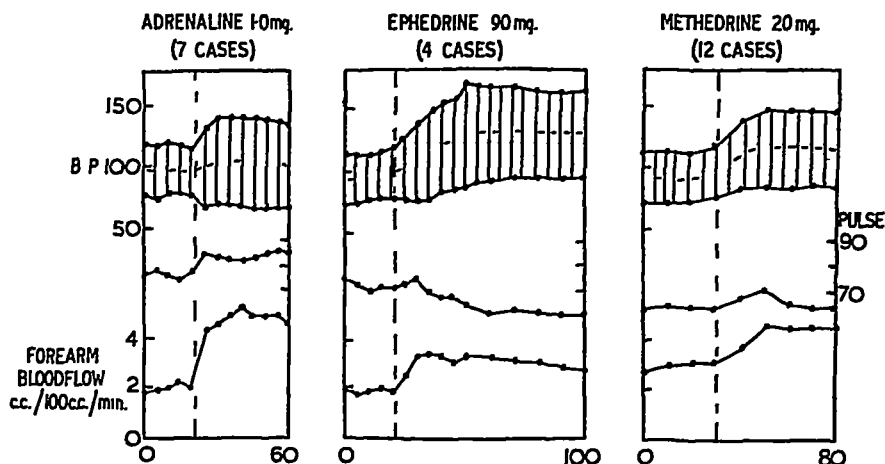
Fig 1   Composite curves for intramuscular injection of adrenaline, ephedrine and methedrine in normal subjects   In each case, from top to bottom, arterial blood pressure, pulse and forearm blood flow (c c /100c c /min)   Doses and numbers of cases as in figure
In this and subsequent figures time is in mins   and the vertical dotted line marks the injection   The mathematical mean B.P   is also shown in some cases

One hour after the injection the B P had begun to fall, and the heart rate and forearm flow were still elevated, and palpitation and tremor persisted

In a few cases intramuscular doses of 0 25 mg   adrenaline were tested The symptoms and circulatory effects were of the same nature as those following the 1 0 mg   dose but were much less pronounced

It seems, therefore, that ordinary intramuscular doses of adrenaline raise the systolic blood pressure, lower the diastolic or leave it unchanged, accelerate the heart to a small degree and cause a considerable increase in the blood flow in the forearm   These results agree with those of Kunkel, Stead, and Weiss (11) and Abramson (1) who used subcutaneous injections

Ephedrine   In a similar series of experiments intramuscular injections of 60 mg   and 90 mg   ephedrine hydrochloride were used (Fig 1)   The action of the substance began later than that of adrenaline, and the full effect was not attained until 30 minutes after administration   Some palpitation was always produced   Mental stimulation was noticed by some subjects but was never great   Skin colour changes were slight or absent , there was an occasional complaint of cold shivers   The action of the drug extended over several hours   The systolic pressure was invariably considerably raised, and the diastolic generally so but occasionally unchanged and, rarely, slightly depressed for a short while   Heart rate changes were

small and irregular, the composite tracing showing that the larger dose produced a protracted decrease of about 10 beats a minute   As in the case of adrenaline the forearm blood flow varied irregularly but was always increased, though to a smaller extent than when adrenaline was used

Thus intramuscular doses of 60 mg and 90 mg ephedrine hydrochloride differ in effect from those of 1 0 mg adrenaline in that the systolic pressure is usually more greatly increased, the diastolic pressure is either raised or unchanged, and is scarcely ever lowered, the heart tends to be slowed rather than quickened, and the forearm blood flow is increased to a smaller extent than when adrenaline is used

Methedrine   The dose used was 20 mg in each case   This quantity of the substance always induced strong mental effects and, if at night, insomnia   One subject remained sleepless for 15 hours after an experiment , another had no difficulty in sleeping three hours after receiving a total of 40 mg   The composite curve from twelve cases is shown in Fig 1 Scrutiny of the individual results revealed the following points

(a) Systolic pressure readings were always increased, the magnitude of the rise varying from case to case   The first rise began at an average of 6 minutes after injection and the maximum was reached after 16 minutes   The elevation was maintained for several hours

(b) Diastolic readings were either unchanged, or increased to a smaller extent than was the systolic

(c) The heart rate varied in an unpredictable manner but tended to show a certain parallelism with the forearm flow which was increased in all cases to a very variable degree, both from one subject to another and in each subject during the course of each experiment

The use of atropine   Adrenaline, and similar compounds, acting directly on the heart tend to accelerate it, yet in all the previous experiments any rise in heart rate which occurred was small in most cases   This is well known to occur in animals (12)   To test whether the smallness of the acceleration was due to vagal restraint, experiments were carried out in which an intramuscular or intravenous injection of 2 0 mg atropine sulphate preceded the injection of the drug concerned   At first the intravenous route was chosen because the appearance of the action was almost instantaneous, but intramuscular injection was later used because the effect was more constant and continuous when so administered, though the maximum acceleration of the heart was not reached until after 30 minutes or more   However it was easier to superimpose the additional effect of one of the drugs on the plateau type of curves following the intramuscular atropine than on the descending slope which follows the initial strong effect of an intravenous dose
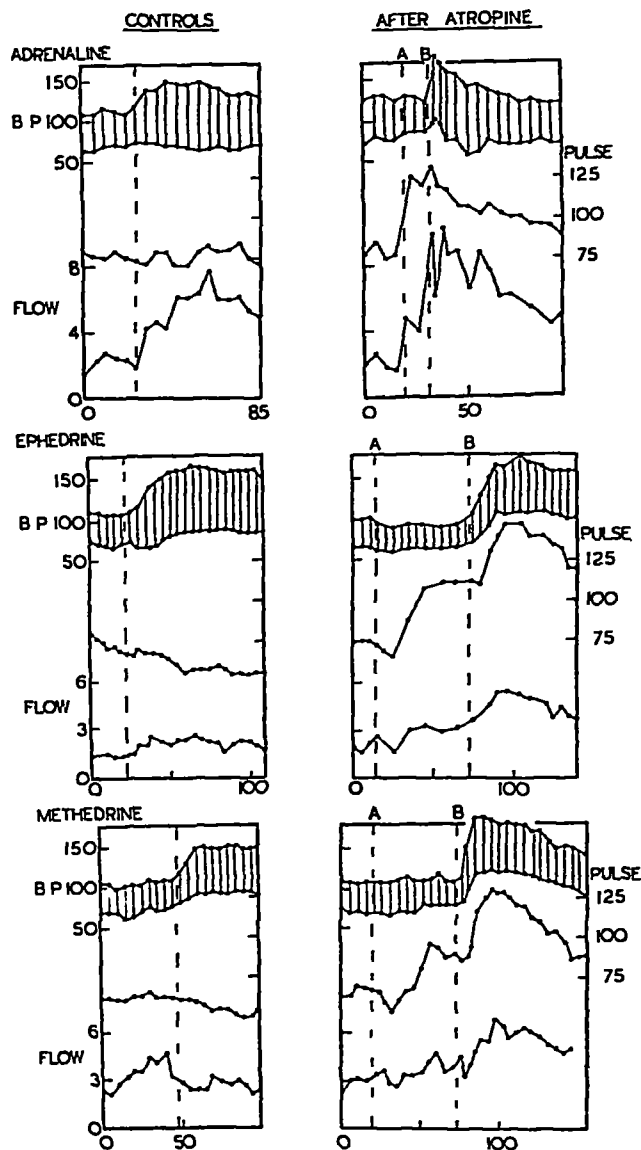
Fig 2 Intramuscular injection of adrenaline, ephedrine and methedrine after atropine Normal subjects In each case from above down, B.P pulse and forearm blood flow

In the left hand graphs control injections of 1 0 mg adrenaline 90 mg ephedrine and 20 mg methedrine were given On the right 2 0 mg atropine sulphate intramuscularly or intravenously at A in each case, followed by the previous doses of adrenaline, ephedrine and methedrine (except in the case of adrenaline where 0 5 mg was given)

Fig 2 shows the results of one experiment from each series, with control experiments in which atropine was not used   An intramuscular injection of 0 5 mg adrenaline after intravenous atropine had a temporarily greater pressor effect than 1 0 mg adrenaline alone, and the heart rate remained at the high level of 125 per minute induced by atropine   Adrenaline alone increased the forearm flow from 2 0 to 6 0 c c per minute   Atropine increased the flow from 2 0 to 4 0 c c , and subsequently 0 5 mg adrenaline further raised the flow to nearly 10 c c per minute   Two other similar experiments gave the same type of result

The effect of atropine on the ephedrine action was more dramatic Injection of 90 mg ephedrine hydrochloride produced a rather greater rise of arterial pressure, particularly diastolic, when preceded by atropine than when given alone   Whereas ephedrine alone slowed the heart of the subject concerned, it quickened it greatly after atropine   As in the case of adrenaline the forearm flow was increased over and above the small rise which followed the intramuscular injection of atropine   Four more experiments gave almost identical results

When methedrine was tested subjects were chosen who had shown a protracted slowing of the heart after a 20 mg dose   Three such experiments were performed, one of which is shown in Fig 2   Atropine affects the response to methedrine in the same way as it does the ephedrine response It increases the pressor effect, both systolic and diastolic, the heart rate rises instead of falling and the forearm flow is increased   The remaining two experiments yielded very similar results

It seems, then, that in man adrenaline, ephedrine and methedrine acting directly on the heart all tend to accelerate it, but that in most individuals the vagus tone is sufficient to permit only a small increase in rate or to produce an actual slowing   Atropine in 2 0 mg doses effectively removes this restraint   The freedom that the heart now experiences is probably the main cause of the greater pressor result   The implications of the increased forearm flow will be discussed later

Hand blood flow

The facial pallor produced by adrenaline suggests that the skin flow should be reduced   The hand does not become as pale as the face but is by far the most convenient site for study   In Fig 3, No 1, the composite results are seen from 5 experiments on different individuals in whom the hand flow was measured when the subjects were comfortably warm and the bath temperature 32°C   The originally rather small hand flow remained practically unchanged by 1 0 mg adrenaline intramuscularly   Pallor of the face occurred in all cases   Apparently adrenaline has little or no influence on hand blood flow when this is already small

To induce a higher resting flow several experiments were carried out, in which one foot was immersed in water at about 42°C   This was sufficient
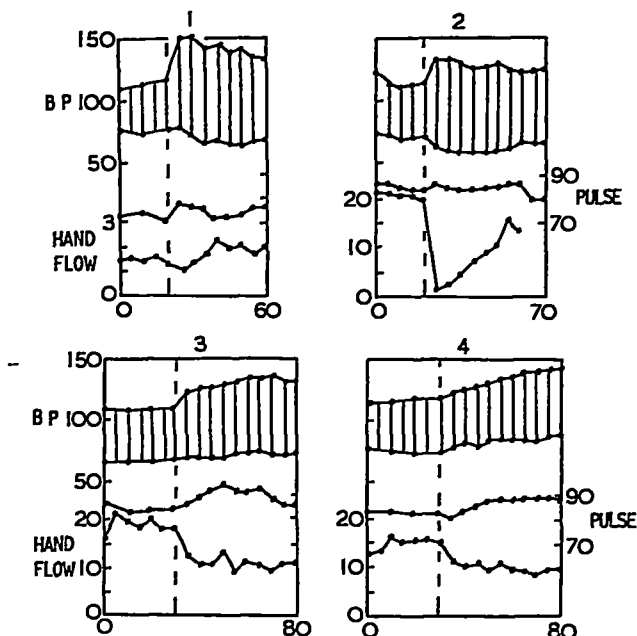
Fig 3 Hand blood flow, B P and pulse in the following circumstances
    No 1   1 0 mg adrenaline I M   Composite from 5 cases
    No 2   As above with indirect heating as described in text   From 3 cases
    No 3   90 mg ephedrine I M , with indirect heating   From 5 cases
    No 4   20 mg methedrine I M , with indirect heating   From 4 cases

to lead to general warming of the skin, but sweating was rarely produced
Greatly increased hand flows resulted, but they were apt to fluctuate during
the period of heating before a steady state was reached   In Fig 3, No 2,
in which three experiments were averaged, the heating had been in progress
for 40 minutes before the start of the recordings shown   The fall in hand
flow which 1 0 mg adrenaline now produced was dramatic, though short-
lived   Kunkel, Stead and Weiss (11) also found that adrenaline greatly
reduced hand flow only when the initial flow had been raised by heating
They attained that end by heating the hand itself to 43°C

In view of the fact that the skin vasoconstriction due to adrenaline
shows itself only when the vessels are first dilated the trials with ephedrine
and methedrine were made under these conditions   Five subjects were
investigated using 90 mg ephedrine hydrochloride and four using 20 mg
methedrine   Again in these experiments heating was in progress for 40
minutes before the recordings were begun   It should be noted that the
hand flow is erratic and variable when high levels are obtained   It is
only by averaging several results that relatively smooth curves are obtained
Just as adrenaline had little effect when the hand was not heated so also

methedrine did not reduce the hand flow in two out of three cases in which heating was not applied
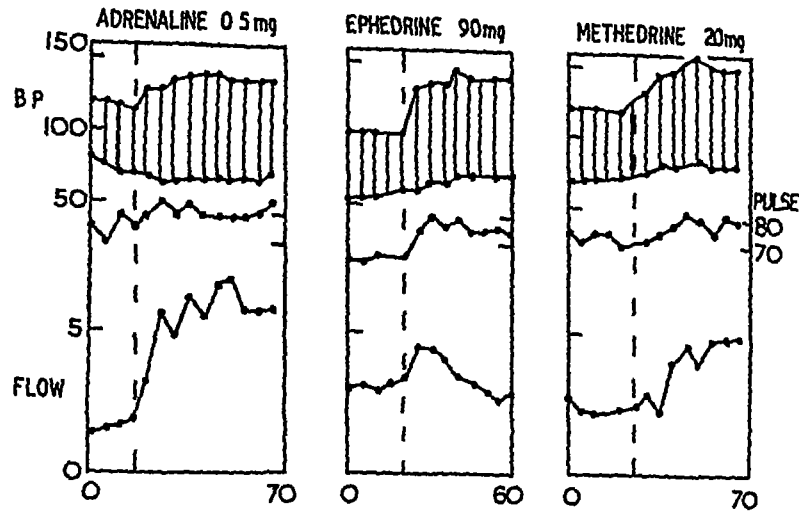
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Fig 4   Intramuscular injection of adrenaline, ephedrine and methedrine in sympathectomized subjects   In each case B P , pulse and forearm blood flow   Doses as shown in figure

Action after sympathectomy   The results are shown in Fig 4   The doses used are indicated in the figure   The response to intramuscular adrenaline was essentially the same as in the normal forearm, as was that to ephedrine   In normally innervated forearms methedrine tended to produce such variable effects that the sympathectomized cases studied cannot be regarded as abnormal   Seven other experiments with adrenaline, eight with ephedrine and four with methedrine yielded very similar results, but since the completeness of the sympathectomy was not investigated in all these cases the observations may not be reliable   No deductions are warranted with regard to possible variations in sensitivity to the drugs following the different types of operation or as compared with the normal forearm

Discussion

The extensive reduction in hand flow produced by adrenaline is in sharp contrast with the increased flow observed in the forearm   The difference is explained by the preponderance of skin in the former and of muscle in the latter   It is interesting to find that adrenaline has little measurable effect on the skin flow when this is already small, whereas its constrictor action becomes progressively more obvious with increased resting flows   Kunkel, Stead and Weiss (11) also found that initially high skin flows were needed if adrenaline was to induce a convincing reduction   Even when the subject is relatively cool as when small hand flows are obtained, the face pales when adrenaline is injected and presumably suffers a decreased

blood flow, but the hand flow under such conditions is little affected   The probable explanation is that the skin of the head is normally much warmer than the extremities and probably has a much larger resting flow and so can be affected by adrenaline at times when the hand flow is already too small to be influenced   In agreement with this is the slight change in the colour of the hand compared with the face when the subject is not deliberately heated   Hertzman (7) using a photo-electric method found that the various parts of the hand approached or even exceeded the forehead in vascularity when in a warm atmosphere (80°F)   The skin vasoconstrictor power of ephedrine and methedrine is definite but feeble compared with adrenaline   In keeping with this the skin colour changes are negligible or absent when these drugs are used

The fact that muscle is the principal constituent of the forearm and skin the main component of the hand explains the opposite action of adrenaline in these sites   The recorded increase in muscle flow and decrease in skin flow probably underestimate the real changes which occur because there is some skin on the forearm and some muscle in the hand

The increase in muscle flow appears to be due mainly to an active dilatation of the muscle vessels   In Fig 1, 1 0 mg adrenaline raises the mean B P * by only 8%, but the forearm flow by 150%   Similarly, 90 mg ephedrine hydrochloride raises the mean B P by 36% and flow by 75%   The composite curve for the methedrine experiments, though perhaps not reliable, shows a 26% rise in mean B P and a 50% rise in forearm flow

Thus all these substances seem to be able to bring about an active dilatation of the blood vessels in skeletal muscle but, as with the action on the skin, adrenaline is the most effective   Even if the figures do not indicate true active dilatation they demonstrate a graded response

Correlation of results   Doses of adrenaline and ephedrine comparable to those used here increase the cardiac output and apparently decrease the total peripheral resistance, adrenaline producing the larger effect in both instances (4, 5, 16)   The results here described suggest that skeletal muscle is probably the main site of this decreased resistance   The greater dilatation observed with adrenaline than with ephedrine agrees with the greater decrease in total peripheral resistance after adrenaline reported by the above workers, and is probably a factor in the lowering of diastolic pressure by adrenaline while ephedrine raises it   The redistribution rather than pressor function of adrenaline is again emphasised by this correlation   In general the results show a very ready dilatation of muscle vessels by adrenaline, a much smaller dilatation by methedrine and an intermediate action by ephedrine

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* The mathematical average of systolic and diastolic readings is used here   it gives an approximate estimate of the true mean

The heart rate changes can also be related to the factors discussed above. Adrenaline brought about a large increase in forearm flow and some acceleration of the pulse (Fig 1), whereas 90 mg ephedrine slowed the heart and caused a much smaller increase in flow Methedrine also produced a small rise in flow and no change in heart rate Presumably then these substances in those doses which strongly dilate muscle vessels, lead to a considerable lowering of peripheral resistance and allow some acceleration of the heart, while no acceleration, or actual slowing, is found when the muscle dilatation is small This relationship explains the parallelism of heart rate and forearm flow observed particularly with methedrine but, to some degree, with all three drugs That the heart rate restraint is vagal, and secondary to the peripheral conditions, is shown by its release by atropine Throughout the results it may be observed that, in general, the diastolic blood pressure is normal or low when muscle flow is high and *vice versa*

The persistent elevation of forearm flow during continued intravenous adrenaline infusions (2) probably corresponds to the same effect found during the steady absorption of an intramuscular injection

The excessive increase in muscle flow following the use of atropine along with one of the other drugs may be due partly to the greater blood pressure which occurs, but a more probable explanation is that if the flow *after* atropine is regarded as the resting value for that subject another drug subsequently increases this in the same ratio as the true normal flow is increased by the same dose, without atropine

In the absence of information on the effects of these drugs on other organs it is impossible to say whether skeletal muscle is the only tissue which experiences vasodilatation and which leads to a reduction in the total peripheral resistance Except when changes are very great the variations in skin blood flow probably have a negligible effect on the total circulation

From a practical point of view, neglecting the duration of action, those substances, such as ephedrine and methedrine, which do not bring about much increase in muscle flow would seem to be more effective pressor agents than those which cause a large active vasodilatation, of which group adrenaline is an example

SUMMARY

1   The effects of intramuscular injections of adrenaline, ephedrine and methedrine on the rate of blood flow in the human forearm and hand have been investigated using the venous occlusion plethysmograph

2   In doses of 1 0 mg , 60 to 90 mg and 20 mg respectively, all these drugs appear to induce active dilatation of the blood vessels of skeletal muscle, adrenaline being the most active in this respect and methedrine probably the least

3   In the same doses these substances do not constrict the skin vessels of the hand when the blood flow is initially low but do so when the initial flow is high, adrenaline again being the most active and methedrine probably the least

4   There is considerable vagal restraint of the heart when these substances are used, it can be removed by atropine

5   Apparently normal responses are obtained in sympathectomized forearms, suggesting that in all cases the action is largely, or entirely, peripheral

6   The blood flow changes in muscle and skin are discussed in relation to the general circulation

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THE MEASUREMENT OF BONE OPACITY

By E G L BYWATERS *

(From the Department of Medicine, British Postgraduate Medical School)

ONLY gross degrees of bone rarefaction are recognisable by visual inspection of roentgengrams   even then, it requires an expert to avoid the numerous fallacies inherent in this assessment   A method has therefore been devised for the quantitative measurement of radio-opacity which avoids subjective judgment and yet can be used in routine hospital practice   This has been employed during the last eight years in following changes of bone density in cases of rheumatoid arthritis   It has many applications in research, since analysis can be made of the variations from part to part, as well as from time to time   It is therefore thought that a description of the method and a discussion of the difficulties and precautions necessary to avoid error might be useful to workers in many fields, experimental, orthopædic and endocrinological

Method

The principle of the method is that there should be exposed at the same time and on the same film as that on which the required bone or limb is to be photographed, a series of solid ivory cylinders of graded thickness   The opacity to light of the resultant images is then measured by a photo-electric cell

(1)   The ivory cylinders are set in a metal holder, are of 12 mm diameter and of a thickness varying by 1 mm from 1 mm to 18 mm   A thick lead disc also is exposed at the same time as the ivory cylinders and the limb

(2)   The reading device (Fig 1) consists of a selenium barrier-layer photo-cell (2 × 4 cm), set on a square wood block 1 6 × 10 × 10 cm   it makes contact (a) with a copper wire outlining the periphery on the deep surface and connected thereby to one terminal of a mirror galvanometer (resistance 350 ohms with a maximum deflection of 6-7 micro amps)   (b) with the other terminal of the galvanometer by a spring clip on its outer

WHITE PAPER

SELENIUM PLATE

COVER SLIP

COPPER WIRE

2 VOLT BULB

SPRING CLIP
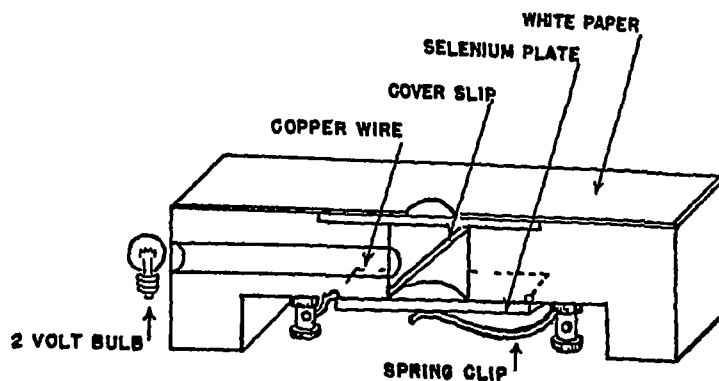
SECTION THROUGH CENTRE OF MEASURING BOX

Fig 1   Diagram of construction of photo electric opacity meter   Scale 1 cm = 1 in

surface, holding it in place   The centre of the photo-cell plate forms the
base of a 16 mm diameter hole bored through the 16 mm thickness of the
wood   The top of this chamber is covered by a 3 × 1 in glass slide inset
in the wood block   on the under surface of the slide is pasted black paper
in which is punched a central hole 9 mm in diameter   The upper surface
of the wood block and glass slide is covered with white paper except for a
corresponding 9 mm diameter hole   This white surface, by reflecting light
through the roentgengrams to be measured, aids accurate localisation   A
further aid to localisation has been found necessary for the more opaque
areas , inside the chamber, whose walls are blackened to avoid reflection,
a thin No 1 glass cover slip is placed at an angle of 45° with the incident
light   A horizontal hole, 4 mm in diameter, bored from the edge of the
wood block into the central chamber allows light from a small 2 volt bulb
outside to impinge on the upper surface of the cover slip and to be reflected
up through the glass slide on to the overlying roentgengram

(3)   A 60 or 100 watt lamp, pointolite type, equipped with a switch
and iris diaphragm travelling vertically on an upright rod, is then clamped
at any required height above the photo-cell aperture   The upright lamp,
rod and the photo-electric cell are both attached to a common base , it is
an advantage for one or the other to be movable out of alignment so that
with dense films, the area of roentgengram to be read can be localised by the
reflected light source described above, and then moved back again to the
same position   Between the lamp and the cell is an iris diaphragm enabling
light intensity to be adjusted more finely than is possible by moving the
source lamp on its upright rod

(4)   Each roentgengram must first be calibrated   An ivory cylinder is
chosen, the density of whose image is less than the range required   This
image, or that of the lead disc, is put over the aperture and the intensity

of the light source adjusted to obtain a maximum galvanometer deflection
The zero point of the galvanometer (*i e*, no deflection) is adjusted with no
light striking the selenium cell   We have found some difficulty in using
the mains current as a source, since there are at times small fluctuations in
intensity   We have therefore used a 12 volt accumulator   Galvanometer
deflections for the remaining ivory cylinders are then read and plotted
against cylinder height, either direct or, if a straight line curve is desired,
after mathematical manipulation

(5)   The deflections for the bone areas to be measured are then read
without altering the light source, and finally the first ivory cylinder deflection
is again checked lest there should have been variation in light intensity
The density of the selected areas is then read off on the calibration curve in
mm of ivory

Theory

Three processes are involved in reading the opacity produced on the
X-ray film by this method, each of which can be represented by a simple
mathematical relationship —

1   The transmission of chemically-active radiation through ivory   and
bone

$$r = R\,e^{-\mu h}$$

where r = transmitted and R = incident radiation in roentgen units,
μ = specific absorption coefficient of ivory and h = the height of the ivory
cylinders in cm   This relation is strictly valid only for monochromatic
X-rays, but is sufficiently accurate for the heterogeneous beam used

2   The production of optical opacity on the film by this transmitted
radiation

$$d = D\,(1 - e^{-\Sigma r})$$

where d = resultant specular film density, D = the greatest density obtain-
able on that film under the given developing conditions, Σ = a constant
denoting intrinsic sensitivity of the film used and r as in 1

3   The transmission of light through the resultant photographic film
and the deflection of a galvanometer thereby produced

$$d = \frac{x}{100} = \log\frac{I^\circ}{I}$$

where x is the deflection of a galvanometer scaled to read extinction
coefficients, I° the incident and I the transmitted light   Thus the
galvanometer deflection can be converted directly to terms of optical density
(d) which, in turn, can be represented in terms of h, the height of the ivory
cylinders by means of combining equations 1 and 2

Eliminating r between these two equations, we get

$$d = D \left\{ 1 - e^{-\Sigma Re^{-\mu h}} \right\}$$

or rearranging,

$$e^{-\Sigma Re^{-\mu h}} = \frac{D - d}{D}$$

Taking logarithms of both sides, we reach

$$\log \left\{ \frac{D - d}{D} \right\} = -\Sigma Re^{-\mu h}$$

or $\log \left(\dfrac{D}{D - d} \right) = \Sigma Re^{-\mu h}$

Taking logarithms again,

$$\log \left(\log \frac{D}{D - d} \right) = \log \Sigma R - \mu h$$

Since for the standard factors used, D, Σ, R and μ are all *constant*, this is a straight line relationship between h and $\log \log \dfrac{D}{D - d}$ (using logarithms to any base)

The height (h) of the ivory cylinders can thus be calculated from the equation

$$h = A - B \log \left(\log \frac{D}{D - d} \right)$$

where $A = \dfrac{\log \Sigma R}{\mu}$ and $B = \dfrac{1}{\mu}$ (using Napierian logarithms)

For the films and quality of X-rays used, Σ will be about 7, R between 0 5 and 1, D = 3 to 4, and μ approximately 4 While it is perhaps more desirable theoretically to have a straight line relationship between height of cylinder and function (galvanometer deflection), as outlined above (and for this purpose it is necessary to use the density of unexposed, *i e*, lead-covered, X-ray film) this involves the use of logarithmic tables, and is perhaps too complicated for ordinary use The calibration curve obtained by direct plotting of cylinder height against galvanometer deflection is as accurate and less time consuming, and has been used for most of these studies An illustrative calibration curve obtained by direct plotting is illustrated in Fig 2A and is compared with the straight line (Fig 2B) obtained on semilog graph paper, using f (deflection) $= \log \dfrac{D}{D - d}$ D being taken as 4 0
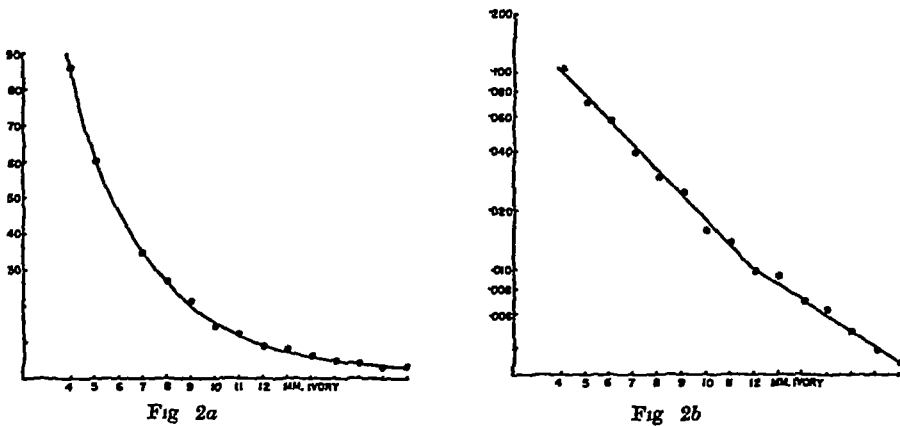
Fig 2a   Fig 2b

Fig 2   Calibration curves —

    (a) direct plotting of cylinder height against galvanometer reading

    (b) Log log $\dfrac{D-d}{D}$ (see test)

Applications to particular problems

For any particular bone area, the factors governing the production of the photographic image have first to be considered so that the most effective choice can be made   Since most of our work has been on the hand, we will deal with this as an example   The factors concerned are —

    1   The type of film

    2   The source of roentgen rays (homogeneity of wavelength or kilovoltage)

    3   The exposure

    4   The distance from the source

    5   The developing solution

    6   The development time

    7   The development temperature

It was thought probable that as the calibrating discs and the selected bone areas are both subject to all the above factors, that variations in any or all of their factors would not be reflected in the final figures   Except for the homogeneity of source rays, which will be discussed below, this is so only in so far as the calibration curve is of satisfactory shape over the range of densities on which it is required to make observations   In the hand and wrist, the range of density varies between 3 and 10 mm of ivory   As the optimum factors for measurement are also those necessary for obtaining clear detail in the roentgengram, the radiologist's judgment is the most useful guide to the factors   The following standardisations were made for the hand —one particular X-ray machine was used throughout at a distance

of 25 inches, using 45 kilovolts, 25 milliamps and 4 seconds for the standard film and latterly 46 KV, 25 ma and 2½ seconds for an ultra speed film (Kodak Blue Ground) Development was for 5 minutes at 65°F, using a metolhydroquinone developer (Kodak D 19b) Even with the procedure standardised as above, considerable variation in position of the curve, although not in shape, occurs on consecutive films This must be due to factors which cannot be standardised in routine hospital work, e g , variations in output of the machine due to intrinsic or extrinsic causes, changes in the developing solution with time and minor fluctuations of the temperature of development It is not felt, however, that these variations affect very greatly the final figure Proof of this is given by six readings of a normal second metacarpal head taken under the above routine conditions at daily intervals, which averaged 5 48 mm ± 0 13 mm S D Deviations from the mean greater than ± 5% would therefore seem to be of significance

Another possible source of error in serial X-ray work is choosing for measurement, each time, exactly the same area Such errors are not, however, difficult to avoid Ten consecutive measurements of the same area of metacarpal head give a galvanometer deflection of between 43 5 and 44% each time The reproducibility of the measurement is well seen in Fig 3 showing no significant local osteoporosis following an impacted Colles fracture
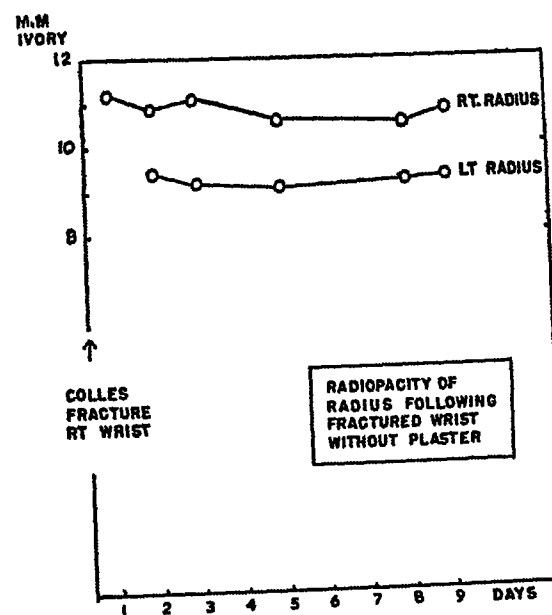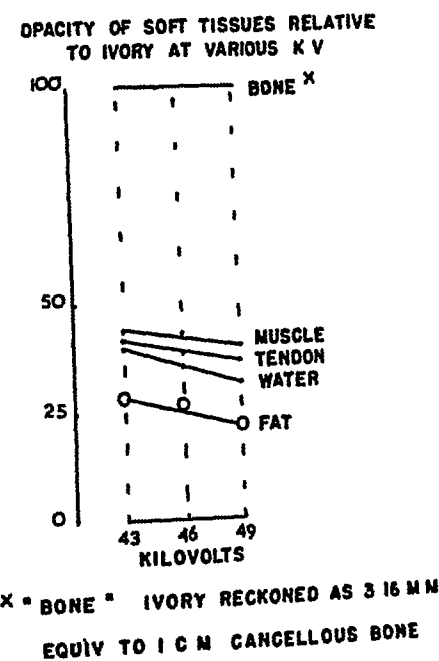

Fig 3

Fig 4

Fig 3   Bone opacity following impacted right Colles fracture, showing no significant osteoporosis

Fig 4   Opacity of various tissues relative to ivory with 3 different kilovoltages

Another source of error is that variations in soft tissue density or thickness may be recorded in the final figure In the fingers, this was not found to be a serious source of error The thickness of soft tissue above and below the metacarpal heads and proximal phalangeal bases was measured in lateral X-rays, with the skin surface marked by a lead paint-line, and the thickness of the space between metacarpals 2 and 3 by the same method The opacity of these masking soft tissues calculated from their thickness on the basis of the metacarpal-space opacity and thickness, amounts to 1 35 mm of ivory per cm compared with 3 16 mm/cm for the bone Thus 3 15 cm of soft tissue (in metacarpal space II-III) = 4 28 mm ivory Soft tissue above and below the metacarpal head measures 1 39 cm = 1 88 mm ivory (by proportion) Whole thickness of bone and soft tissue here = 6 42 mm ivory , therefore bone alone (1 48 cm thick) = 6 42 — 1 88 mm = 4 54 mm of ivory Thus of the total density here, $\dfrac{4\ 54 \times 100}{6\ 42} = 71\%$ is due to bone

The maximum increase in circumference of the fingers in a severe case of rheumatoid arthritis is + 1 2 cm , amounting to + 0 4 cm in diameter This would add 0 53 mm to a total density of 6 42 mm , i e , about + 8% * In areas of the body where soft tissues are more preponderant, it would probably be difficult, if not impossible, to allow for soft tissue opacity since, besides the effects of scattered radiation, the mass absorption coefficient of any substance depends on the wavelength of incident light (*see* Fig 4) With the usual heterogeneous beam, photographically active wavelengths transmitted through mixed tissue would not theoretically be comparable in resultant density with those transmitted through the ivory cylinders For this reason, the use of aluminium wedges instead of ivory or bone would give erroneous results

Summary

A quantitative and objective method for measuring bone opacity is described and discussed it involves the simultaneous exposure of a graded series of radio-opaque cylinders and the plotting of a calibration curve for each separate X-ray film This method is capable of use in a routine hospital department It has been found useful in following bone rarefaction during the course of an illness, and makes possible an analysis of the mechanism of bone rarefaction in local and general conditions of ill-health

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* This calculation assumes that tissue causing swelling is of the same radio-opacity as normal a more detailed study of the relative opacity of different soft tissue by this method has not yet been completed

THE ABSORPTION OF XYLOSE IN STEATORRHŒA

By L P R FOURMAN

(From the Nuffield Department of Medicine, University of Oxford)

FAT and glucose are thought to be phosphorylated during absorption from the small intestine (21) whereas xylose is probably not (*see* Höber, 1945, for summary of evidence (9)) Verzar (20) and more recently Stannus (19) have suggested that the sprue syndrome may be due to a failure of phosphorylation, and on this theory one might expect xylose to be normally absorbed in sprue It is relatively easy to study the absorption of xylose in the human subject This pentose sugar goes through the liver unchanged (5) and is excreted by the kidney rapidly, though some is destroyed in the tissues The amount absorbed from the intestine can be calculated by comparing the amounts excreted in the urine after oral and intravenous administration (13) When the sugar is given intravenously, the rates of excretion and of destruction in the tissues are both proportional to the plasma concentration, and the total amount excreted represents a constant fraction of the amount injected (4) In man, this proportion is about 40% when renal function is normal (13) and when xylose is taken by mouth one may assume that the kidneys excrete a similar proportion of the xylose which has been absorbed from the small intestine The blood xylose curves return to near normal 5 to 6 hours after ingestion so that normally practically all of the excretion of the sugar occurs during this time (5)

Xylose has previously been used to measure absorption from the small intestine by Helmer and Fouts (8) They were studying pernicious anæmia, but also reported impaired absorption of xylose in two cases of idiopathic steatorrhœa In the present work on the absorption of xylose in steatorrhœa, the technique of Helmer and Fouts was followed with some modification Twenty-five grams of d-xylose in about 450 ml or one pint of water, that is, in approximately isotonic solution, were given to the fasting subject The amount of xylose excreted in the urine during the following five hours was measured The sugar was estimated by the method of Schaffer and Hartmann (quoted by Peters and van Slyke, 1932 (17)) after precipitation of the non-sugar reducing substances (22) No attempt was made to get rid of the fermentable sugars in the urine because their quantity is comparatively negligible In estimating the reagent blank a sample of the fasting urine was included, and the blank titration value did not vary significantly from case to case It was found that over 24 hours there was

no destruction of xylose added to clean urine in a concentration of 0 5%
Some precaution should however be taken to preserve the urine since it
was found that in a culture of intestinal organisms, which may contaminate
urine, xylose is destroyed   During the collection periods urine was kept
in a refrigerator

The absorption of xylose was studied in three patients with tropical
sprue, three patients with idiopathic steatorrhœa, and also in two patients
with healed tuberculous mesenteric adenitis   All the patients had
steatorrhœa as shown by an abnormal amount of fat in the stools on
chemical analysis and none of them showed any evidence of pancreatic or
biliary disease   None of the patients had watery diarrhœa   Radio-
logically, intestinal motility was normal and renal function was normal as
measured by the urea clearance or by the specific gravity test

TABLE I

Five hour xylose excretion, after 25 grams of xylose by mouth, in grams

	Normals	Tropical sprue	Idiopathic steatorrhœa	T B mesenteric adenitis
	6 7	1 05	2 1	3 3
	6 5	3 0	2 5	1 5
	4 3	1 6	1 4	—
	6 7	—	—	—
Mean	6 0		2 1	

Results

The five hour xylose excretion figures are shown in Table I   The normal
values, mean 6 0 g , were slightly higher than those found by Helmer and
Fouts in eight subjects (range 4 26-5 33 g , mean 4 68 g)   The results from
the patients with steatorrhœa were all low, and there was no significant
difference between the three types of case   In several patients low values
were again found after a period of treatment with liver and yeast extract
or folic acid, although at least in the patients with tropical sprue this
treatment had produced a clinical remission (Table II)   Since there was a
possibility that the low five hour urinary xylose excretions might have been
due to delay in gastric emptying, though on the radiological evidence this
was unlikely, the xylose excretion was measured over 24 hours in three
patients (Table III)   The findings indicate that a delay in absorption does
in part account for the low values found, since an appreciable amount of
xylose was excreted by the patients after the end of the five hour period,

TABLE II.

Changes in xylose absorption with treatment

Disease	Treatment	Duration of treatment	Five hour xylose excretion (in grams) after 25 g by mouth	
			Before treatment	After treatment
Tropical sprue	Liver and yeast extract	21 days	1 0	1 8
Tropical sprue	Folic acid	14 days	1 6	1 2
Idiopathic steatorrhœa	Folic acid	14 days	2 1	1 8

TABLE III

Comparison of 5 hour and 24-hour xylose excretions

Disease	Xylose excretions, in grams	
	5 hour	24-hour
Idiopathic steatorrhœa	1 38	3 09
T B mesenteric adenitis	1 46	2 37
T B mesenteric adenitis	3 34	3 81

TABLE IV

Five hour xylose excretion compared with fat absorption
(per cent of intake, measured over 12 days)

Patient		Fat absorption (%)	Five-hour xylose excretion (grams)
Tropical sprue	After treatment (21 days' folic acid)	77	3 0
	After treatment (2 months' liver and yeast extract)	85	3 0
Tropical sprue	Before treatment	77	1 6
	After treatment	57	1 2
Idiopathic steatorrhœa	Before treatment	85	2 1
	After treatment	77	1 8
Idiopathic steatorrhœa	After treatment	80	2 5

but even when measured over 24 hours the total excretion was below normal
The proportion of xylose excreted by the patients in five hours was variable
This may account for the fact that there was no close quantitative
relationship between the five hour urinary xylose figures and the degree of
impairment of fat absorption as measured in 12 day balance experiments
(Table IV)   A 24 hour rather than a five hour urine collection period might
have advantages in estimating xylose absorption in steatorrhœa   After
intravenous injection, the excretion of xylose was found to be normal, as
determined in one patient   Of 10 grams given intravenously, 4 92 g were
excreted in the following 24 hours , this patient excreted 3 81 g of xylose
in 24 hours after taking 25 g by mouth   This experiment confirms that the
low xylose excretions found were not due to an abnormality of metabolism
or excretion of xylose in patients with steatorrhœa, but to a failure of
absorption

Maegraith (15) has reported improved absorption of glucose in some
patients with tropical sprue, after giving the sugar with a buffered phosphate
mixture   He based his conclusions on the results of blood glucose curves
The effect of phosphate on xylose absorption was tried in one patient with
tropical sprue   Four grams of a mixture of mono- and di- sodium phosphate
(pH 7) were given with the xylose, increasing the amount of water to maintain
an approximately isotonic solution   The five hour excretions were, without
phosphate, 3 g , with phosphate, 2 7 g , so that in this experiment the
phosphate had no beneficial effect on absorption

Discussion

It is interesting to note that the patients with mesenteric adenitis
showed the same absorption defect for xylose as the patients with sprue
and idiopathic steatorrhœa, in whom no anatomic lesion is likely to be
found   Since there is no reason to suppose that xylose is absorbed through
the lacteals, lacteal obstruction probably does not alone account for the
absorption defect in these patients   Possibly the failure of fat absorption
interferes with the cellular function of absorbing sugars, or it may even be
that the same kind of physiological lesion is responsible for the absorption
defect of both idiopathic steatorrhœa and sprue on the one hand and
mesenteric adenitis on the other   How that lesion is produced and what the
nature of the fault is, whether nervous, humoral, or enzymatic, remains
unknown   Evidence that has been put forward for a phosphorylation defect
in sprue is inconclusive   The effect of phosphate on glucose absorption in
sprue (15) is not necessarily related to the phosphorylation process, since the
acceleration of glucose absorption by phosphate in animals (16) has been
shown to be due to the buffering effect of the phosphate and is reproducible
by other buffer mixtures (11)   It has been claimed (14) that the finding of
normal fructose curves after the ingestion of sucrose in sprue is evidence of
normal absorption of fructose in this disease, but other evidence indicates

that fructose absorption is also impaired in sprue (6) It is questionable, however, whether or not fructose is phosphorylated during absorption Other substances which are poorly absorbed in steatorrhœa include iron (3) and several water-soluble vitamins (2) The present work shows that xylose is poorly absorbed in steatorrhœa This sugar is not absorbed by simple diffusion, not only are different pentoses absorbed at different rates (13) but d-xylose is much more rapidly absorbed than l-xylose in the rat (10) so that molecular configuration as well as molecular size affect its absorption On the other hand, there is no evidence that this sugar is phosphorylated and extracts of rabbit kidney which phosphorylate glucose do not phosphorylate l-xylose (23) Evidence is accumulating that the supposed differences in the absorption of glucose and xylose, on which the phosphorylation theory for the absorption of glucose is partly based, may not be as fundamental as had been thought (18), one may not conclude, however, that because xylose is not absorbed by simple diffusion it is absorbed by phosphorylation The present observations do not provide any support for the theory that sprue is due to a defect in a phosphorylation mechanism, but they do not disprove this theory

Some positive conclusions may be drawn from the finding of impaired xylose absorption in steatorrhœa There is little information on the localization of function in the small intestine such as has been obtained for absorption from the renal tubule, but by an ingenious series of experiments McCance and Madders (13) showed that xylose is wholly absorbed from the upper part of the small intestine, and this has received some confirmation by direct experiment on the dog (12) Absorption from this part at least of the small intestine must therefore be affected in sprue The findings suggest that xylose can be used to measure intestinal absorption in disease This would have definite advantages compared with the difficulties of measuring the absorption of fat or of sugars that are metabolised Fat absorption, to be measured at all accurately, has to be measured over at least a 12-day balance period, not only is this a very tedious procedure, but this technique will not detect any rapid changes in absorption Glucose absorption cannot be measured by the balance technique, because the unabsorbed sugar is destroyed by bacterial fermentation in the bowel, and one has to depend on the interpretation of blood curves to estimate the absorption of this substance Since the shape of the blood curve depends on the rate of removal of the substances from the blood stream as much as on the rate of absorption, this method is unreliable for the study of absorption Fuller investigation may show that the finding of normal xylose absorption provides a simple and rapid means of excluding steatorrhœa Deficient xylose absorption cannot however be taken as necessarily indicating the presence of steatorrhœa since deficient absorption of carbohydrates may occur in other diseases such as deficiency states (7), ulcerative colitis (1) and pernicious anæmia (8)

SUMMARY

The absorption of d-xylose was studied in three patients with tropical sprue, three patients with idiopathic steatorrhœa and two patients with steatorrhœa due to healed tuberculous mesenteric adenitis In all of them xylose was poorly absorbed The significance of this finding is discussed in relation to the theory that sprue may be due to a failure of phosphorylation It is suggested that xylose may be useful for the study of intestinal absorption in steatorrhœa

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RENAL HÆMODYNAMICS IN ACUTE NEPHRITIS

By D A K BLACK, R PLATT, E N ROWLANDS and H VARLEY

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ALTHOUGH the clearance methods devised by Homer Smith have been applied to clinical problems for a number of years, the recorded results have mostly been obtained on patients with essential hypertension, toxæmia of pregnancy, and chronic nephritis   Earle, Taggart and Shannon (2) included in their nephritic series a number of patients who were considered to be in the acute stage of nephritis, but none of these was in the early weeks of the disease   They found that the inulin clearance was depressed to a greater extent than the diodrast, giving low values for the filtration fraction Hilden (7) found that the diodrast clearance in acute nephritis was normal, or only slightly reduced   Urea clearance was relatively low, compared with diodrast clearance , a reduction in glomerular filtration rate was inferred from this, but actual inulin clearances were not done   The present paper gives the results of inulin and diodrast clearances in three patients who were first observed at a stage in type I nephritis when blood was still present in the urine, and the blood pressure was elevated   Further estimations were made during and after recovery

TABLE I
Clearances in acute nephritis

| Patient	Date	Urine volume (ml /min.)	Inulin clearance (ml / min)	Diodrast clearance (ml /min)	Inulin clearance/ Diodrast clearance (Filtration fraction)	Urea clearance (ml /min)	B P (mm.Hg)
1	17 12 46	1 1	60 5	485	0 125	—	150/110
„	17 1 47	1 0	98	580	0 169	—	140/85
„	25 4 47	2·0	76	466	0 163	—	120/80
2	18.2 47	1 8	67	567	0 118	—	135/95
3	26 2 47	0 75	32 5	384	0 090	24 5	165/112
„	12 3 47	0 6	46	405	0 114	26	140/95
,	11 4 47	1 3	115	640	0 180	53	115/85
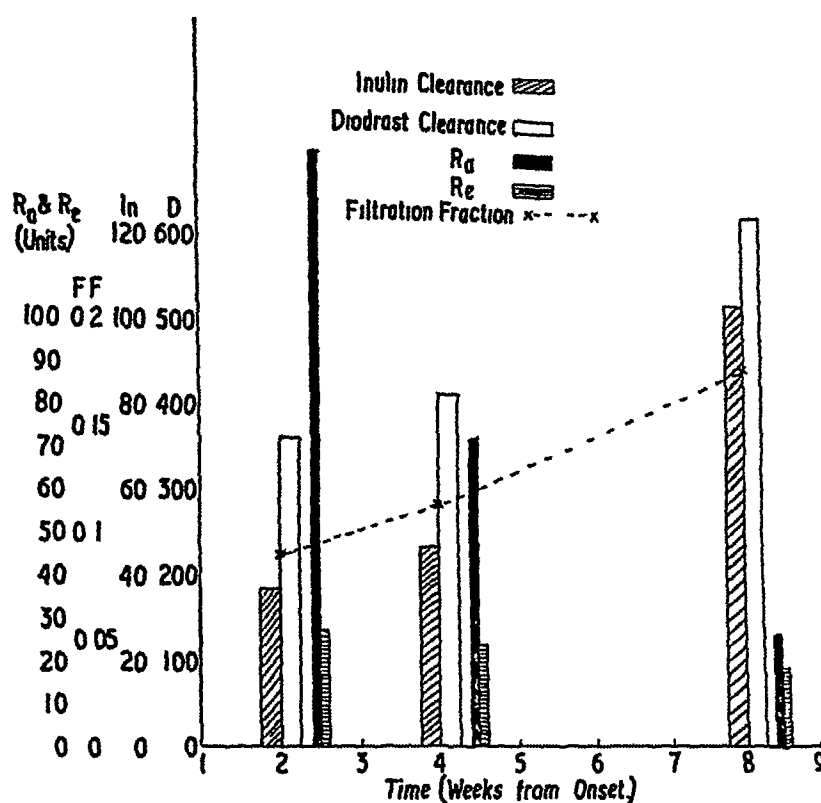
Fig 1 Clearance results and calculated values for afferent and efferent arteriolar resistance in Case 3 The diodrast clearances are on a scale 1/5 of that of the inulin clearance, so that with a " normal " filtration fraction of 0 2 these columns would be of the same height

Methods and results

Clinical data on the three patients studied are given in an appendix The results (Table I, Fig 1) are based in each case on three 20-minute clearance periods, the urine being obtained by catheter, with bladder washout A continuous inulin-diodrast infusion by intravenous drip was maintained at a rate of 0 5 ml/min, after a priming infusion Fluid intake was not forced either before or during the collection period, with the method of collection used, agreement between urine samples was adequate

The clearance results, together with the calculated filtration fraction, are shown in Table I During the acute stage of the nephritis, the inulin clearance was lowered below the normal range in all three patients With recovery, the inulin clearance rose in the two patients on whom serial observations were made The diodrast clearance was much less reduced in the acute stage, so that the ratio of inulin to diodrast clearance (the filtration fraction) was notably lowered The filtration fraction rose to within normal limits in the two patients on whom follow-up estimations were made

Interpretation of results

The figures of Earle, Taggart, and Shannon (2) show that there is no significant reduction in tubular excretory mass (diodrast T_M) at a rather later stage in the course of acute nephritis   If this is also true at the stage of acute nephritis in which we first examined our patients, diodrast clearances in them can be taken as representing the effective renal blood flow   Similarly, inulin clearance is taken to represent the amount of glomerular filtrate formed per minute   On this basis, the low filtration observed during the hypertensive phase of acute nephritis indicates that the amount of glomerular filtrate formed is small in relation to the amount of blood flowing through the kidney   Our present knowledge of renal function suggests three possible explanations for this phenomenon —

(1)   The permeability of the glomerular membrane is reduced

(2)   Part of the blood-flow through the kidney is " shunted," so that it fails to perfuse the glomeruli

(3)   There is an alteration in the relative tone of the afferent and efferent arterioles, such that glomerular capillary pressure is lowered, while blood-flow is maintained at a nearly normal figure

These possibilities may now be discussed

1   *Decreased permeability of the glomerular capillary wall*   Histologically, the glomerular lesion in acute nephritis is a proliferative inflammation involving the glomerular capillary epithelium   The thickened capillary wall might seem to be the structural equivalent of diminished permeability, and Earle, Taggart and Shannon (2) advance this as the explanation of the low filtration fraction which they found in subacute and chronic nephritis   It seems to us, however, that diminished glomerular permeability cannot be the explanation of a low filtration fraction in acute nephritis , for inflamed capillaries in general are more permeable than normal, and the constant presence of both albumin and red cells in the urine of acute nephritis is difficult to reconcile with diminished glomerular permeability

2   *Presence of a " shunt "*   Although the important work of Trueta, Barclay and their colleagues (13) has shown that in the rabbit it is possible for blood to be diverted from cortex to medulla, it is uncertain how far this occurs in man   Before yielding to the temptation of applying these recent results to our own problem, we must consider more fully what would have to be the characteristics of a " shunt " which would account for a low filtration fraction   Not only must the glomerular blood-flow be diminished, but the tubular flow must be maintained, to account for the relatively high diodrast clearance   Now, it is not certain whether the medullary circulation brought into play in the Trueta experiment does give adequate tubular perfusion , their observation that the renal vein blood becomes red suggests that tubular perfusion may not be adequate   If a large part of the medullary

blood-flow goes by the vasa recta, it is again difficult to picture the convoluted tubules as being effectively perfused   Diversion through a vessel of the Ludwig-Isaacs type would give tubular perfusion, but the frequency and importance of such vessels in the human kidney are very much in doubt (6) A further argument against the activity of a shunt mechanism in acute nephritis is that the shunt observed in animals is associated at times with renal vein pulsation, suggesting a low renal vascular resistance, whereas the data presented in our next section indicate that renal vascular resistance is increased

3   *Alteration in renal afferent and efferent tone*   The general relationship between blood-flow, vascular resistance and perfusion pressure in an organ is derived from Poiseuille's law, and can be expressed as $Q \propto \frac{P}{R}$ when Q is blood flow, P is perfusion pressure, and R is the total resistance to flow offered by the vessels , it is assumed that the viscosity of the blood leaving the organ is the same as that of the blood entering it   This may also be expressed as $R \propto P/Q$ In acute nephritis, the perfusion pressure, which depends on the systemic blood pressure, is increased, while the renal blood flow is somewhat diminished   The total resistance offered by the renal vessels is therefore increased   If decreased glomerular permeability or a shunt mechanism can be excluded, and if there is no obstruction to the outflow of urine, a low filtration rate must depend on a reduction of the effective filtration pressure inside the glomerular loops   The question arises, what type of alteration in renal vascular tone would produce a fall in intracapillary glomerular pressure at a time when blood in the renal artery is at a pressure greater than normal   The answer can only be, constriction of the afferent arteriole out of proportion to whatever tone may be present in the efferent arteriole   Lamport (8, 9) has presented formulæ for the calculation of afferent arteriolar resistance (R_A), and for efferent arteriolar resistance (R_E), which in the 1941 paper includes also the further resistance offered by the tubular capillaries   The formulæ we have used are —

$$R_A = \frac{P_M - P_0' - 40}{HD}$$

$$R_E = \frac{(1 - 0\ 47\ F)\ (P_0' - P_0 + 10)}{HD}$$

(P_M = mean arterial pressure HD = renal blood flow, calculated from the plasma percentage, as determined by hæmatocrit, and diodrast clearance P_0' = osmotic pressure of plasma leaving the glomerulus P_0 = osmotic pressure of plasma entering the glomerulus F = filtration fraction)

We have used the 1941 formulæ (8) as our present purpose does not require the differentiation of efferent arteriolar resistance from the resistance

in the tubular capillaries and venous channels   For details of the derivation of these formulæ, Lamport's own papers should be consulted   Their use involves certain assumptions, which are probably not universally true, but which are likely to be as valid in the kidney of acute nephritis as in the normal kidney, so that a comparison may be usefully made, even though the resistances are relative rather than absolute values   To justify our use of the formulæ in this rather pragmatic fashion, we may mention the main assumptions involved —

1   That blood flowing through the small kidney vessels behaves in general conformity with Poiseuille's law   Although Poiseuille's law is based on experiments with a homogeneous fluid in rigid capillary tubes, it has been shown that blood-flow in an isolated limb behaves in somewhat the same way, and a correction can be applied to Poiseuille's law (as is done in Lamport's formula) to make it more nearly applicable to blood flowing through natural vessels

2   That filtration equilibrium is attained in the glomerulus   The validity of this assumption for the normal kidney has been shown by Smith *et al* (12), and their arguments should apply equally to the kidney in acute nephritis

3   That the osmotic pressure of plasma can be calculated from the total protein content of plasma   Some error is certainly involved here, in view of the differing albumin   globulin ratios in different patients ,   this

TABLE II

Calculated afferent, efferent, and total renal arteriolar resistance

Patient	Date	R_A (units)	R_E (units)	R_T (units)	R_A/R_E
1	17 12 46	76	20	96	3 8
,,	17 1 47	34	20	54	1 7
,	25 4 47	33	24	57	1 4
2	18 2 47	44	17	61	2 6
3	26 2 47	138	26	164	5 3
	12 3 47	73	24	97	3 0
,,	11 4 47	26	19	45	1 4

NOTE —The   units   of resistance referred to are those used by Lamport (8) and are given by him as 1000 × mm Hg per c c per min   From data of Smith *et al* Lamport has calculated R_A and R_E in seven normal subjects, the average values being

R_A 18 8   (Range   6 9 — 37 5)

R_E 18 2   (,,   11 0 — 25 9)

R_A/R_E 1 04   (,   0 6 —   1 5)

error will be less if the results in the same patient are compared at different times

4   The other minor assumptions involved are justified by Lamport (8) The results obtained when Lamport's formulæ are applied to our data are shown in Table II, together with average normal values given by Lamport It will be seen that the total resistance is increased during the early hypertensive stage of acute nephritis, and falls towards normal with recovery   The afferent resistance is increased much more than is the efferent, as may be most clearly seen from the R_A/R_E ratio   This is the type of change required to account for diminished glomerular filtration, in the presence of an increased renal artery pressure

Discussion

It is tempting, in view of the evidence of renal arteriolar constriction in acute nephritis, to suppose that the renin mechanism may be brought into play, and so account for the rise in blood pressure in this disease   The general picture of renal hæmodynamics does not, however, give much support to this view   When hypertensin is injected into a normal human subject, a rise of 40 mm mercury in systemic blood-pressure is associated with a 50% fall in renal blood-flow, and a considerable rise in the filtration fraction (10)   The renal action of hypertensin, like that of adrenaline, would seem to be exerted mainly on the efferent arteriole , and it is of course the efferent arteriole which is mainly affected in essential hypertension (5) In our patients, however, the low filtration fraction indicates that constriction is on the afferent side of the glomerular circulation , while the application of Lamport's formulæ indicates very definite increase in afferent arteriolar resistance, there is no definite increase in efferent arteriolar resistance Moreover, the renal blood flow figures are higher than would be the case in a normal person who was given hypertensin in a dose sufficient to produce the degree of systemic hypertension observed   The relatively normal blood flow also makes it doubtful whether the renin mechanism would be set in motion in this type of renal disease   Our results in fact demonstrate a state of affairs in the kidney which would not be expected either to give rise to or to result from release of renin ,   and it is interesting to note that Pickering (11) concluded from a study of heat elimination from the hand that the hypertension of acute nephritis was not of the same mechanism as in essential hypertension and chronic nephritis and suggested that it was neurogenic rather than humoral   On the other hand, Goldblatt (3) in his review of the subject affirms that renin has actually been demonstrated in the systemic blood in human cases of acute glomerulonephritis with hypertension   The only paper which we have been able to find claiming that renin is demonstrable in acute glomerulonephritis refers to a patient with a blood pressure of 260/160, no clinical details being given ,   and it is further stated that renin could not be demonstrated in other patients with acute

nephritis (1)   This evidence seems somewhat equivocal, but if later work should show that renin is in fact released in acute nephritis, we can only assume that the kidney in this disease is unable to respond to renin in a normal manner, so that the expected constriction of the efferent arteriole fails to occur   In the meantime, our results as they stand support the view of Pickering that hypertension in acute nephritis is not due to the renin mechanism

SUMMARY

1   In three patients in the acute stage of type I nephritis, with elevation of the systemic blood pressure, inulin and diodrast clearances showed a greatly reduced glomerular filtration rate and a moderate diminution in renal blood flow, so that the filtration fraction was reduced below normal limits   In two of the patients, estimations made when the blood pressure had returned to normal showed a normal filtration fraction

2   Reasons are given against the low filtration fraction being caused by decreased glomerular capillary permeability, or by the operation of a shunt mechanism

3   Calculations of the afferent and efferent arteriolar resistance, using Lamport's formulæ, show that the afferent resistance is greatly increased during the acute hypertensive phase of the disease, while the efferent resistance remains within normal limits   The low filtration rate is therefore considered to be caused mainly by a fall in glomerular filtration pressure

4   The significance of these results is discussed with relation to the mechanism of raised blood pressure in acute type I nephritis

Appendix of case records

Case 1, female, aet 48   No previous renal disease   Admitted 7 12 46, four weeks after a sore throat, and two weeks after the onset of œdema of face, hands and legs   On admission, oliguria with blood and albumin in the urine, œdema of face, hands and legs   B P   180/115   Blood urea 44 mg /100 ml   The urine was sterile on culture, and contained many blood, leucocyte, and epithelial casts   On an intake of 20 oz of fluid daily, the patient remained oliguric, passing 10-15 oz of urine daily, until 21 12 46, when a moderate diuresis set in, and the œdema disappeared   She was discharged from hospital well on 27 1 47, with a B P of 140/85, and with no albumin in the urine   At follow-up examination, however, on 24 7 47, she was found to have a blood pressure of 145/95, and albumin in the urine

The first clearance records were obtained on 17 12 46, when she was still œdematous, with a urine volume of 15 oz and a B P of 150/110   The second set of clearances was done on 17 1 47, when her œdema had gone, her daily urine volume was over 30 oz , and her B P 140/85, but a trace of albumin was still present in the urine   A third set of results was obtained on 25 4 47

Case 2, female, aet 35   Had frequent colds, with sore throat, during the two months before her admission on 13 2 47   One week before admission, swelling of the face and ankles was noticed, together with headache and dyspnœa   On admission, there was oliguria, and the urine contained red cells and albumin   The B P was 170/110, and œdema of face, hands, and legs was present   Blood urea 24 mg /100 ml   Diuresis set in soon after admission, and she was discharged well on 7 3 47, with a B P of 125/80, and no albumin in the urine

The clearance records were obtained on 18 2 47, when the B P was 135/95, and œdema was still present   This patient could not attend for follow-up estimation after recovery

Case 3, female, aet 16   Admitted 25 2 47, one month after the onset of an unusually severe cold, which lasted two weeks   Dull pain in the loins, hæmaturia, oliguria and œdema of the face had been present for two weeks before admission   At the time of admission, the urine contained blood and albumin, and there were hyaline and epithelial casts   Oedema was not prominent   B P was 165/112, blood urea 20 mg /100 ml   Recovery from her attack of acute nephritis was slow, and although the blood pressure had come down to 140/85 on 4 4 47, and she was free from all symptoms, the urine still contained 2 5 g of albumin per litre when she was discharged on 14 4 47

The first clearance records were obtained on 26 2 47, her B P then being 165/112, and urine volume for that day 25 oz   The second set of clearances was done on 12 3 47, the B P being 140/95, and urine volume 19 oz   The third set of clearances was done on 11 4 47, with B P 115/85 and urine volume 43 oz   on 10 4 47

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SLOW RECOVERY FROM ISCHÆMIA IN HUMAN NERVES *

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WHEN the circulation to the human arm is arrested, sensation becomes impaired in the fingers after about ten minutes By half an hour, sensation is defective over much of the forearm and certain voluntary movements have become paralysed If the circulation is then released, these defects are found to disappear within a few minutes, and the arm appears normal when tested

If such an experiment is repeated on different days, these changes develop after times which are about constant in the same subject If, however, two such occlusions of the circulation—each lasting, say, half an hour—are done on the same arm in one day, it has long been known that a given change may be observed a few minutes earlier in the second occlusion than in the first (4), even though the circulation to the arm has been free for a period of hours between the two occlusions For this reason, in studies on sensory and motor changes during circulatory occlusion in this department, repeated occlusions of the same arm within the same day have ordinarily been avoided It seemed desirable, however, to study the recovery from ischæmia in a quantitative way for several reasons It was not clear to what nerve function so slow a recovery could be ascribed In addition, if the effects of ischæmia could still be demonstrated after a period of hours, it was possible that paresis in certain clinical conditions might be related to a cumulant effect of quite short but frequently repeated periods of circulatory obstruction Experiments were therefore undertaken in which the circulation was occluded by means of a pneumatic cuff on the upper arm until paralysis of a recorded voluntary movement had occurred The circulation was then restored for a measured interval of time, and the occlusion repeated, the duration of ischæmia required for paralysis in the second occlusion being compared with that necessary in the first

Method

A sphygmomanometer cuff of which the rubber bag measured 12 cm by 34 cm was wound round the upper arm in a marked position on it, ordinarily such that the proximal edge of the bag was 60 cm from the tip of the middle finger. To avoid congestion of its vessels the arm was raised above the head before arresting the circulation. The cuff was inflated rapidly by the admission of air from a 6-gallon bottle, at a pressure between 160 and 180 mm of mercury. The limb was then immersed in water at 35°C to the upper border of the cuff. The temperature of the water and of the skin under the cuff were recorded with thermocouples, and maintained constant.

Although sensory changes were timed and noted, it was thought desirable to rely upon a more objective method of determining nerve paralysis. Movements of opposition of the thumb were therefore recorded on a kymograph at intervals during the occlusion. This action was selected because it gave a clear endpoint of paralysis which did not involve an unduly long occlusion. During ischæmia, the hand assumes a position of rest in which the fingers are slightly flexed at all joints, while the thumb is somewhat abducted from the palm but is not flexed. The movement recorded was such that from this position the tip of the thumb moved towards the tip of the fourth finger, without flexion at any thumb joint. It did not prove difficult to prevent flexion of the thumb or other movements interfering with the endpoint. The contractions were made at times announced by the experimenter, starting about ten minutes after occlusion of the circulation, the exact time being unknown to the subject. Contractions were then made ¼-minutely until the subject himself announced them as becoming weak, after which they were made ¼-minutely until they failed to give a recorded or visible movement of the thumb. In this way, the onset of paralysis could be timed with reasonable accuracy, and ischæmic muscle pain did not occur in any of the 102 such occlusions performed. The amplitude of contraction was recorded, using a hinged rod which rested against the medial aspect of the metacarpo-phalangeal joint of the thumb. The movement of this rod, which was 21 cm long, compressed a rubber bulb which recorded by means of a tambour. Recording was roughly isotonic.

Under these circumstances, the amplitude of contraction usually remained about constant for approximately 20 minutes from circulatory arrest, after which it fell rapidly and regularly until contraction was too weak or too localised to be recorded (Fig 1). Several contractions could then usually be observed by inspection of the thumb itself before the movement was finally lost. A record was made of the time at which these ¼-minutely voluntary efforts first failed to produce a visible contraction. This measure of paralysis time was preferred to the time at which the amplitude of contraction fell to, say, half its initial value, since this time, although it could be accurately determined from the kymograph record,

was affected by any differences in position of the hand relative to the recording apparatus in different occlusions

In subjects who were familiar with testing sensation during circulatory arrest, records were made of the rate of spread of numbness and of anæsthesia up the arm   Attention was confined to changes affecting the skin of the dorsum of the thumb and of the radial border of the forearm The experimenter, using a clock which was invisible to the subject, recorded the times at which the latter announced that numbness or anæsthesia had extended over each phalanx of the thumb and over various fractions of the forearm above the wrist   Numbness was interpreted as the earliest sensory defect to testing of the skin with a normal finger , anæsthesia corresponded to complete loss of sensation to light touch with similar testing
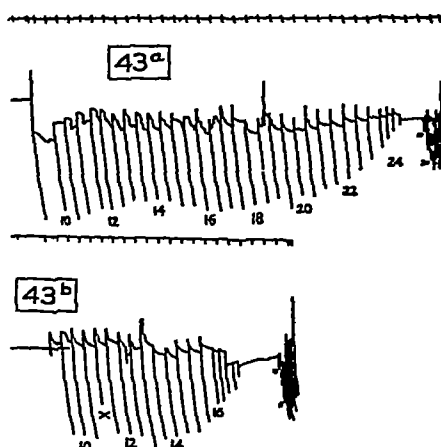

Fig 1   Amplitude of thumb contractions in a control occlusion (43a) and in a second occlusion (43b) following after a 32 min. period of free circulation.
    Time marker, half minutes   Figures record time in minutes since occlusion   Release of circulation after 25 minutes in 43a and after 18½ minutes in 43b   Subsequent contractions attempted at 5 second intervals until 50 seconds after release

The subject had no clue from the clock or kymograph drum, as to the times at which sensation or movement were becoming paralysed   The failing movements followed a regular pattern of subjective weakness, decreasing amplitude, slowed and tremulous development, localised contraction, and final failure   We were satisfied that the endpoint was not appreciably influenced by variability in the effort made to contract the muscle, and that the subject was uncertain as to whether failure was occurring earlier or later than in any previous or control occlusion   Sensory changes occurred at rather less constant times in different control occlusions, and it is difficult to be sure that criteria of numbness or anæsthesia did not vary It is likely that such variability occurred, since in certain control occlusions

the times for all sensory changes in the arm were systematically somewhat earlier, and in other cases later, than normal For this reason, times of motor paralysis are considerably more reliable and have been used as the basis for determinations in the present work, although the times of sensory changes have supported the same conclusions as given by those of motor paralysis

In each experiment, the circulation was released when the movements of the thumb had become paralysed—ordinarily at the ensuing half-minute Contractions were then attempted every 5 seconds for 50 seconds after restoration of the circulation, the first one usually failing to record and that at about 30 seconds commonly reaching the full amplitude recorded early in occlusion

Except when a second occlusion was to start within a few minutes, the cuff was then removed and its actual position during occlusion measured using the upper border of reactive hyperæmia to indicate the level to which the skin had been rendered ischæmic by the cuff

Results

The general course of recovery from circulatory occlusion was studied first in two subjects, and the influence of certain experimental errors investigated The validity of the data was then extended by observations on three other subjects unfamiliar with the previous results, on whom the time of paralysis was established after recovery periods of 4 minutes, 1 hour, and 24 hours Finally, tests were made to determine any cumulant effect and the site of action of the phenomena studied The consistency and errors of control observations will be discussed first

Control data In the pairs of circulatory occlusions, the first, or control occlusion, was never done on an arm which had been occluded during the previous 5 days Under these circumstances, paralysis occurred within reasonably constant times which had an average of 24 7 minutes in the five subjects (Table I), and a standard deviation of 1 1 minute for repetition on any given subject's arm The time of paralysis did not appear to be related to the interval since the preceding occlusion on the same arm when this was 5 days or more Sensory changes had a rather lower constancy of onset in control occlusions, the time at which they were noted at different levels having a standard deviation of 1 9 min in the two subjects in whom these changes were regularly recorded (*see* Table II)

Since it has been shown that the onset of paralysis is affected by the position of the cuff on the arm (4), this position, and the skin temperature under the cuff, have been measured and controlled in all experiments In addition, the effects of their variation were separately studied in a subsidiary experiment on two subjects in whom the effect of a 24-hour recovery interval between occlusions was also being examined Sixteen occlusions were

TABLE I

Paralysis times in control occlusions

Subject	Arm length Olecranon to 3rd finger tip (cms)	Top of Cuff Mean distance to 3rd finger tip (cms)	Paralysis times (min.)	Mean time (min)	Mean time as for cuff at 60 cms (min)
E D B	47	63 0	23¾, 23½, 24¼ 23, 26¼, 26½	24 6	25 5
E E P	48	62 3	23½, 22½, 23½, 24½, 22¾ 23¾ 24¾, 24	23 7	24 4
H H W	45	59 9	25½, 27½, 27¼, 28¾	27 3	27 3
A J H	44	59 5	21¾, 21½, 22½, 22½	22 1	22 0
J H H	46	60 0	24¼ 22¾, 25½, 24¾	24 4	24 4
			Mean for all subjects		24 7 min
			Standard deviation of means		1 9 min
			Standard deviation of single determinations in the same individual		1 1 min

TABLE II

Mean times for sensory defects in control occlusions (min)

Defect	Numbness		Anæsthesia	
Subject	E.D.B	E E P	E D B	E E.P
Position				
Thumb tip	10 2	9 9	19 3	16 8
„ distal joint	12 1	10 6	20 5	17 6
, proximal joint	12 8	11 7	21 2	18 4
Wrist	16 5	13 3	22 7	19 2
¼ up forearm	18 6	13 6	23 3	19 6
½ up forearm	19 2	14 9	—	21 5
Mean standard deviation of single observations (min)	2 3	0 6	1 3	2 2

performed, each subject undergoing 4 occlusions before, and 4 after a 24-hour interval of free circulation   In each of these groups of 4 occlusions, paralysis times were determined for a high and for a lower cuff position, each position being combined both with a warm and a cooler arm temperature (*see* Table III)

In this way the 16 occlusions give eight separate comparisons between pairs of occlusions differing only in the position of the cuff on the upper arm , and 8 estimates are similarly available for the effect of arm temperature, for the difference between the subjects, and, as will be described later, for the

TABLE III

| Subject | Expt | Control occlusions | | | Expt | Occlusions 24 hours later | | | |
		Mean Cuff Temp (°C)	Cuff height from R₃ tip (cm)	Paralysis time (min)		Mean Cuff Temp (°C)	Cuff height from R₃ tip (cm)	Paralysis time (min.)	Difference in Paralysis time (min)
E D B	20(a)	**34 8**	56 7	24½	20(b)	**35 5**	58 1	26½	+2
	22(a)	31 0	56 5	24	22(b)	30 7	57 1	28	+4
	24(a)	**35 0**	**65 0**	20½	24(b)	**34 6**	**64 3**	23½	+2¾
	28(a)	31 7	**65 0**	22½	28(b)	32 9	**65 0**	24½	+2
E E P	21(a)	30 5	**68 4**	21¼	21(b)	30 7	**68 1**	23½	+2¼
	23(a)	**35 2**	60 5	21	23(b)	**35 0**	59 4	25¼	+4¼
	25(a)	31 2	59 7	23½	25(b)	31 4	59 6	28	+4½
	29(a)	**34 4**	**68 2**	21½	29(b)	**34 6**	**68 0**	25¼	+3¾

Mean increase of paralysis time in later occlusion +3 2

Standard error 0 4

NOTE.—Warmer cuff temperatures and higher cuff positions are indicated by heavy type

TABLE IV
Effect of cuff position and temperature

Subject	Expts		Difference in cuff position (cm)	Difference in paralysis time (min)	Expts		Difference in cuff temp (°C)	Difference in paralysis time (min)
E D B	20a	24a	+8 3	—4	20a	22a	+3 8	+¼
	22a	28a	+8 5	—1½	24a	28a	+3 3	—1½
	20b	24b	+6 2	—3¼	20b	22b	+4 8	—1½
	22b	28b	+7 5	—3½	24b	28b	+1 7	—1
E E P	21a	25a	+8 7	—2¼	29a	21a	+3 9	+¼
	23a	29a	+7 7	+½	23a	25a	+4 0	—2¼
	21b	25b	+8 5	—4¼	29b	21b	+3 9	+1¼
	23b	29b	+8 6	0	23b	25b	+3 6	—2¼
Mean difference			+8 0	—2 3			+3 6	—0 9
Significance of mean differences			0 3 min /cm				0 25°C /cm	

n = 7   t = 38   P = <0 01

t = 17   P = 0 14

effects of a 24-hour recovery interval   It was found (*see* Table IV) that in these subjects, neither cuff position on the upper arm, nor arm temperature as judged by skin temperature below the cuff during occlusion, is an important

source of error in the present experiments An alteration in cuff position by 8 cms down the arm resulted in an average delay of paralysis by 2 3 minutes Since cuff position during occlusion, as measured from the area of reactive hyperæmia, did not usually vary by more than 1 cm in different occlusions, the error from this cause should be under ¼ minute Similarly, paralysis is probably delayed slightly when skin temperature is low, a mean delay of 0 9 minute corresponding to a temperature fall from 35 0°C to 31 6°C It is clear that thermojunctions on the skin give little indication of the temperatures of nerves lying deeply in an ischæmic arm These skin temperatures were however usually within 1°C in different experiments following similar procedures, and variations in this factor should not ordinarily have caused errors of over ¼ minute It is possible that, in re-occlusions after very short periods of release, reactive hyperæmia may warm the deep tissues of the arm and cause a slightly earlier time of paralysis than the skin temperatures would indicate , but it seems unlikely that appreciable error would result even here, since the arm was normally warm before the control occlusion also

These sources of error are thus adequately controlled, since their effect is within the observed variability of paralysis times It may be noted that the variability of the repeated estimates of the effect of a particular factor given in Tables III and IV indicate a standard deviation of 1 5 minute for the difference between paralysis times, which corresponds to one of 1 1 minute for each paralysis time, agreeing well with the variability in control times noted above
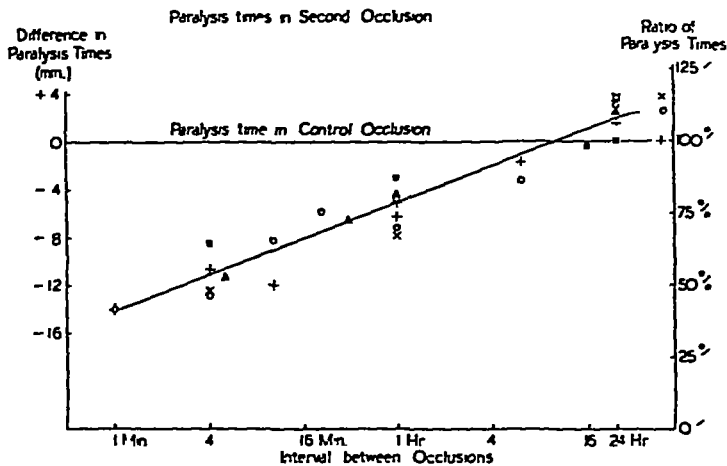

Fig 2 Paralysis times in second occlusions plotted in terms of those in control occlusions preceding them by various intervals with free circulation Different symbols represent different subjects Data do not include the confirmatory results of Table IV

Recovery from ischæmia When the circulation is arrested twice on the same arm, with various intervals of free circulation between the occlusions, paralysis occurs earlier in the second than in the first occlusion by an amount shown in Fig 2 Here, the difference in the times of paralysis is plotted against the duration of free circulation between the occlusions, using a logarithmic scale for the latter for convenience of presentation It will be seen that recovery is incomplete for at least 6 hours after an occlusion of about 25 minutes and sufficient to paralyse opposition Although the time needed for paralysis returns rapidly to half its normal value after the first few minutes of a restored circulation, it only reaches three-quarters of normal by about 30 minutes, or seven-eighths of normal by 2 hours of circulatory re-establishment The rate of recovery is broadly similar in different subjects (Table V)

TABLE V

Paralysis times in different subjects (minutes)

Subject	Interval between occlusions								
	4 min			*1 hr*			*24 hr*		
	Paralysis times in								
	Contr occ	2nd occ	Diff	Contr occ	2nd occ	Diff	Contr occ	2nd occ	Diff
E D B	23¾	13	—10¾	23¼	18¼	—5	24¼	26	+1½
E E P	23½	10¾	—12¾	23¼	16¼	—7¼	24¼	27	+2¾
H H W	25¾	13	—12¼	27¼	19¾	—7¾	27¼	31¼	+3¾
A J H	21¾	13¼	— 8¾	21¼	18¼	—3	22¼	22¾	0
J H H	24½	13¼	—11¼	22¾	18¼	—4½	25¼	27¾	+2¾
	(5 min interval)								
Mean difference			—11 2			—5 5			+2 0
Standard error			0 8			0 9			0 6

When the course of sensory paralysis is compared in such pairs of occlusions, all the stages of sensory loss are also found to develop earlier in the second than in the first It is of interest that the times at which various defects occur in a second occlusion do not differ from the corresponding times in the first by a constant amount, but are reduced in a roughly constant ratio

For example, paralysis of opposition occurs normally after 25 minutes of occlusion, but would develop after only 60% of this time, or 10 minutes earlier, in a second occlusion after about 7 minutes of

free circulation Yet numbness, which reaches the wrist at, say, 15 minutes in the first occlusion, will do so in the second in 60% of this time, and so 6 and not 10 minutes earlier than in the first This relationship holds as an approximate one both for sensory defects— the arrival of numbness or anæsthesia at different levels in the hand or arm—and also for different degrees of impairment of the amplitude of muscular contraction

It is thus an over-simplification to picture a second occlusion as starting with a certain "handicap," or zero error in time, owing to incomplete recovery from the first The actual time differences, as given in Fig 2 are thus dependent on the choice of thumb paralysis as an index , but the ratio of the paralysis times, as compared with those of control occlusions, will be roughly true for any stage of sensory or motor paralysis studied

It has been stated that muscle contractions rapidly recover, often to full amplitude, within ½ minute of releasing the circulation * For any stage of recovery shortly after release, there is a corresponding time before release at which the amplitude of contraction was equally reduced   Table VI shows the relationship between the interval of free circulation after when a particular amplitude is reached, and the interval of occluded circulation before release at which the amplitude of contraction was equally reduced For recovery times from 10 to 30 seconds, each second of free circulation reverses the effects of about 9 seconds of occluded circulation

TABLE VI

Recovery after control occlusions

Time of contraction after release (sec)	5	10	15	20	25	30
Time before release at which contraction was of equal amplitude (sec)	89	107	138	180	217	240
Ratio (time before/time after)	17 8	10 7	9 2	9 0	8 7	8 0

Data based on 31 recovery curves of 5 subjects

In the two subjects first studied, occlusions were done 24 hours after control occlusions, to determine whether the times of paralysis had then returned to their control values   In both these experiments, paralysis was found to occur rather later than in the corresponding control—or in any other control—occlusion This odd finding was studied in two ways As stated above, the effect of a 24 hour period of recovery was determined in 8 further experiments, which gave the consistent results shown in Table III

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*Since movement was recorded isotonically using a light load, we do not claim to record the earliest defect in muscle power during ischæmia, or the time of full recovery after release

In addition, tests were made on 3 furthei subjects, unfamiliar with the pieceding results, of whom two showed a similai increase of paralysis time after a 24 hour recovery, and one a time equal to the control time (Table V) On poolmg all these tests, the mean mciease was of 2 75 minutes (Standaid Error 0 36 min) on the mean control time of 24 7 minutes It is clear that this difference is larger than could be due to a chance sequence of iandom errors (t = 7 8 foi n = 12 where values of t greater than 3 1 correspond to a chance probability less than 0 01) , and we can see no systematic crroi likely to cause such a result It is known that a reactive hypeiæmia of skin vessels to the control occlusion would have fallen to a low value within thirty minutes of circulatory release (3) While it is possible that reactive hyperæmia in neive might be much more prolonged, it is difficult to believe that this could be the cause of the observations The differences aic altogethei largei than could be caused by a difference in the effort made to move the thumb, the thumb movement having commonly been little, if at all, reduced in amplitude in the second occlusion at a time when it had already failed in the first After the control occlusions the arms showed no œdema oi persistent vascular change, and gave no pain or other sensation causing them to be protected from normal use It seems likely, therefore, that this increased resistance to ischæmia is a genuine phenomenon arising as a phase of nerve recovery

Aftei a 48-houi recovery interval, paralysis times 0 0, +2 5 and +3 8 min greatei than the corresponding control times were observed in three subjects, as compared with mean increases of 2 4, 3 5 and 3 8 min for these subjects at 24 hours, suggesting that the resistance to ischæmia is decreasing at 48 hours after the control occlusion

Cumulant effects Since recovery from a circulatoiy occlusion is not complete for many hours, it seemed possible that the effects of repeated occlusions during such a peiiod might be cumulant If they were, it might be of immediate clinical inteiest in any condition in which intermittent

TABLE VII

Paralysis times in successive occlusions

Subject	Interval between successive occlusions	Successive paralysis times (min)			
		Control	2nd	3rd	4th occlusion
E D B	60 min	26	20	21½	20¼
E E P	60 min	23¾	19	18	17¼
J H H	32 min	24¾	18½	18¾	18¾
	Mean values	24 9	19 1	19 4	18 9
E E P	24 hrs	24	27¾	28¾	27½

pressure on nerve trunks was associated with paresis   Experiments of two types were therefore made on this point

In the first group of experiments, four successive occlusions were each continued until opposition became paralysed, a constant interval of recovery being allowed between them   This interval was of 1 hour in two subjects and of half an hour in another

It is seen from Table VII that paralysis occurs earlier in the second than in the first occlusion by the amount already described, but paralysis develops in the third and fourth occlusion at about the same time as in the second In a further experiment, the occlusions were separated by 24-hour intervals, and the third and fourth occlusions showed no greater increase in paralysis time than was observed in the second (Table VII)   It appears, therefore, that under these circumstances successive occlusions do not have a cumulant effect

TABLE VIII

Paralysis times after cycles of occlusion and release (minutes)

Subject	Cycles		No of Cycles	Paralysis time in ensuing occlusion	Mean Control Paralysis Time	Difference due to Cycles
	Duration Occ (min.)	Rel				
E.E.P	2	4	10	21 5	23 7	(?2 2)
H.H W	3	1	8	26 25	27 3	(?1 0)
E E.P	2¾	¼	10	16 5	23 7	7 2

In the second group, a series of short occlusions, each followed by a short period of release, preceded a long occlusion in which the time required to paralyse opposition was compared with that in control occlusions   Table VIII shows that cumulant effects are evident if the period of release of the circulation is sufficiently short   Thus, appreciable impairment was observed after repeated occlusions of 2¾ minutes, separated by intervals of free circulation lasting ¼ minute, but not after repeated occlusions of 3 minutes with intervals of 1 minute free circulation   A further experiment with alternating occlusion and release indicated that, in one subject, cumulation of the effects of ischæmia occurs if the periods of ischæmia are about 8 times those of free circulation   Thus with alternate occlusions of 2 minutes 40 seconds and releases of 20 seconds, a sensory defect, established by previous ischæmia, had neither advanced up nor retreated down the arm at the end of successive occlusions   The same balance was achieved with 5 minutes 20 seconds periods of occlusion and 40 seconds periods of release   This result is comparable with that mentioned above (p 311) that a short period of free circulation reverses the effects of a preceding period of ischæmia lasting 9 times as long, as judged by the amplitude of muscle contractions   It

appears unlikely that the paresis observed in any clinical condition is due to an intermittent mechanical interference with vascular supply of this type, where the circulation is free for so short a proportion of the total time

Site of action of ischæmia The data of Table IV may be held to indicate that ischæmia is having its effect on nerve trunks under the cuff and not on nerve endings in the hand, since the time of paralysis is influenced by the position of the cuff on the upper arm A more direct proof was required, however, since the slow recovery might still be at the nerve ending which thereby came to limit the duration of thumb movements in the second occlusion, although not having limited it in the first It is also possible that anatomical considerations comparable to those discussed by Weddell and Sinclair (7) might possibly affect the time of paralysis Paralysis of a suitable movement was therefore induced using the clamp described by Lewis, Pickering and Rothschild (4) which presses on and occludes the circulation to nerve trunks while allowing a free circulation to the hand As soon as muscle paralysis had been produced, the circulation was restored for 4 minutes and then re-occluded, now using a cuff on the upper arm, the cuff covering the site previously occupied by the clamp In such experiments, paralysis occurs earlier in this second occlusion using the cuff than in control occlusions with such a cuff, indicating that the paralysis by the clamp had left a state which contributed to the later paralysis by the cuff (see Table IX)

TABLE IX

Effect of control occlusion by clamp producing local ischæmia of nerves

[Subject	Movement	Paralysis time by cuff (minutes)		
		(a) after 4 min release following paralysis by clamp	(b) control— without preceding paralysis	(c) after 4 min release following paralysis by cuff
A J H	Adduction of 5th finger	12	19½	14
E.E P	Flexion of distal joint of index	10	27	11

Further, the time at which the cuff produced paralysis in such experiments following clamp paralysis was similar to that observed in a cuff paralysis following a previous paralysis by a cuff Local pressure causing ischæmia of the nerve trunks, therefore, leaves behind an impairment recovering slowly and comparable with that achieved by the cuff It seems clear, therefore, that the slow recovery described is of nerve trunks and not of nerve endings.

Discussion

The full recovery of nerve from circulatory occlusion has, in these experiments, lasted more than 12, and probably about 24 times as long as the period of occlusion causing it This rate of recovery may seem unexpectedly slow for two reasons Firstly, the rapid restoration, within a minute of release, of approximately normal motor and sensory function* suggests that recovery is rapidly completed after occlusion, unless it is realised that the re-establishment of normal function is a highly insensitive index of complete recovery Secondly, if paralysis is due to a slow exhaustion of oxygen by the low metabolic needs of active nerve, the restoration of an adequate oxygen supply to the nerve should be more rapid when the circulation is released and recovery might be correspondingly swift It has indeed been found that, in the first minute of recovery, each second is sufficient to reverse the effects of about 9 seconds of preceding ischæmia (Table VI) This phase, which may well be determined by reoxygenation of nerve, is sufficient to bring the nerve rapidly to full function on simple testing After the first minute, however, the recovery process becomes greatly slower, and is still in progress six hours later It is hard to believe that, at such periods, oxygenation is still inadequate, and other hypotheses for this slow nerve recovery must be considered

It is possible, in compression of segments of isolated nerve, that œdema or deformation of the nerve at the limits of compression may initially prevent the restoration of circulation when pressure is removed, or damage the nerve in other ways, and Bentley and Schlapp (1) have shown that recovery may be delayed for long periods or indefinitely under these circumstances Comparable results were obtained by Lewis and Pochin (5) from the pressure of a narrow band on the finger, when nerve recovery was delayed for many weeks, presumably because the nerve was damaged irreversibly by deformation If similar narrow bands are used as ligatures on the rabbit's ear, it is found (6) that a zone of œdema borders that of compression, and that arteries may not re open across this zone as soon as compression is removed, while venous flow is often much delayed

It seems most unlikely that, in the present experiments, any such effects were caused in nerve by its local deformation or by any known effects of pressure other than that of circulatory arrest Pressure by the wide cuff used does not cause, even in the skin, sufficient deformation at its limits to delay the immediate re-entry and passage of blood throughout the area In deeper tissues such as the nerves concerned, no appreciable deformation or localised nipping can be expected as the limits of compression must be ill defined This point has been further examined by radiography of the arm before and during compression by the cuff, using fine wires lying between arm and cuff It was found that the greatest change in arm diameter was

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* " Pins and needles,' and probably slight sensory and motor defects persist for longer periods

a 4% reduction at the upper border of the cuff, while at the lower border the change was even less Under these circumstances, the mechanical deformation of a nerve lying deeply in the arm must be very slight It seems clear that the phenomena studied are due to cessation of blood supply to nerve trunks and the surrounding tissues, and that recovery is slow although blood and oxygen supply to the nerves are probably restored immediately

We have not investigated what deficiency or accumulation of any metabolite during circulatory occlusion is responsible for the defect which recovers slowly Certain causes, such as the breakdown of ionic distributions across membranes, would presumably recover rapidly and are unlikely to be responsible Others, such as the destruction or local loss of organic molecules, might be more slowly restored The recent work of Harvey on the slow return of plasma choline-esterase concentrations after di isopropylfluorophosphate (2) shows that the delay in nerve recovery is by no means too long to be explained by a local loss of such materials If these phenomena can be reproduced in experimental animals, any deficiency which delays recovery may be easily identified If so, by returning to human experiment the effects of materials on the speed of recovery could readily be studied for an hour after administration, since the paralysis time is still substantially reduced for this period after a control occlusion It is clear that the apparently increased resistance to ischæmia which recurs 24 hours after occlusion in man can also be more exactly and rigorously examined if reproduced under the fully objective conditions of animal experiment

The clinical implications of this work have not been explored It seems unlikely that paresis from intermittent ischæmia is of general importance since, at least for the durations studied, circulatory arrest must occupy 90% of the total cycle if paresis is to develop rapidly It has not been established whether occlusion for a shorter proportion of the time, but continued indefinitely, would ultimately cause an equilibrium state in which paresis occurred

It is of some interest that, in the hours following a circulatory occlusion, the nerve may be normal to simple tests of function, yet have a consistent decrease of resistance to further occlusion We have not wished at this stage to occlude the circulation to limbs in which the sensory or motor function was impaired, for example in peripheral neuritis or pressure palsy, since the longer lasting effects of ischæmia might be irreversible in such cases It is clearly possible, however, that certain such cases might be due to a sustained impairment of whatever factor causes the defect in recovery after occlusion The duration of ischæmia necessary to paralyse a particular nerve function might, if it could be safely determined, offer a quantitative mode of investigation which would, moreover, detect lesser degrees of impairment in nerves of which the function was apparently normal

SUMMARY

After occlusion of the circulation to the human arm with a cuff for 25 minutes, movements and sensation return rapidly to normal   For a period of many hours, however, full recovery of the nerve can be shown to be incomplete, since in a second occlusion, motor and sensory changes develop earlier than in the first (Fig 2)

Recovery is only complete in this sense after about 10 hours, while at 24 hours after the first occlusion, the nerves consistently have a slightly *increased* resistance to ischæmia, paralysis of thumb opposition occurring at a time greater by 10% than in control occlusions

The slow recovery from occlusion appears to be due to an effect of ischæmia on nerves below the cuff   The effect is not cumulant in a sequence of occlusions unless the periods of free circulation are small compared with those of circulatory occlusion

The possible causes and clinical implications of the slow recovery are discussed

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"ON THE BEHAVIOUR OF DEEP AND CUTANEOUS SENSIBILITY DURING NERVE BLOCKS"

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INTRODUCTION

FOR many years it was recognised that pain arising from the deep tissues, such as the muscles, bones and joints, was in some way different from pain arising from the skin   This distinction was further emphasised by Lewis (10) who showed that the various deep structures all gave rise to a diffuse aching pain which could always be distinguished by the subject from pain arising from the skin by its different and characteristic quality   Because of this Lewis suggested that deep pain and skin pain might be separate modes of sensibility   Since then deep and cutaneous pain have been found to differ in other respects (9, 11) and if it was shown that deep pain and skin pain sensibility could be dissociated the view that deep pain and skin pain are separate modes of sensibility would receive substantial support

It is generally accepted that the tissues deep to the skin may give rise to sensations of pressure as well as pain (8, 19, 20)   Head (7) observed that firm pressure transmitted through an area of anæsthetic skin could still be appreciated as pressure and Von Frey (17) observed that the threshold of pressure sensibility became raised when the skin was anæsthetised   To what extent this deep pressure sensibility which remains after the skin has been anæsthetised should be considered to include joint and position sensibility or the sense of vibration transmitted through bone is not entirely clear, but these sensations are all provoked by mechanical deformation of the tissues and they have a similar non-painful quality   Deep pain, on the other hand, is undoubtedly distinct from deep pressure   Weddell and Harpman (20) found that sensations of pain and pressure were elicited from spatially separate spots in the deep tissues   Lewis and Pochin (14) found that in nerve blocks produced by pressure and ischæmia deep pain sensibility remained after all sense of position and deep pressure had been lost, while in procaine blocks Heinbecker, Bishop and O'Leary (8) found that pressure could still be appreciated at a time when "pressure pain" could no longer be elicited   It is, therefore, probable, as Walshe remarked (19), that there are two modes of deep sensibility —pressure and pain

Not much is known about the manner in which this deep sensibility fails and recovers. Cutaneous pain sensibility is well known to pass through an abnormal phase during nerve regeneration. This abnormal phase has been described under the titles of "protopathic pain" (Head, 7), "hyperpathia" (Foerster, 4) and "enhancement" (Trotter and Davies, 16). In nerve blocks produced by pressure similar changes occur in cutaneous pain sensibility (14), but in nerve blocks produced by cooling and by procaine the findings are somewhat different. It is not clear to what extent deep pain sensibility may pass through a similar abnormal phase, or whether the other modes of sensibility, such as touch, may behave in a similar manner. We have, therefore, repeated the well known experiments of blocking nerves by means of pressure (6, 12, 14), cooling (2, 18) and procaine infiltration (5, 6, 8) and we have studied the behaviour of deep and cutaneous sensibility, paying special attention to abnormalities of sensibility other than sensory loss.

Method

The changes of both deep and cutaneous sensibility were studied along the ulnar border of our own hand during blocks of the ulnar nerve produced by pressure, cooling and procaine infiltration.

Pressure was applied to the ulnar nerve above the elbow by a clamp similar to the one used by Lewis (12, 14) but using pressures of 60 to 70 mm of mercury in a single cuff 16 cm wide placed against the medial aspect of the arm. Procaine blocks were produced by the usual technique of infiltration, gradual onset of paralysis being obtained by a perineural infiltration in the ulnar groove, using 5 c c of 2% procaine solution with the addition of 0 001 g of adrenalin. For cooling we immersed the elbow in an ice salt mixture, the temperature in the ulnar groove being recorded by a subcutaneous thermocouple inserted beside the nerve. As in all our sensory experiments the subject was kept warm and comfortable. The hand to be examined was kept at a temperature of 30° to 35°C by resting it upon a rubber hot water bottle and covering it with a second bottle when testing was not in progress, the temperature being checked from time to time by a thermocouple attached to the skin of the hand.

Deep sensibility was tested on the dorsum of the hand in the fourth interosseous space and over the fifth metacarpophalangeal joint. Cutaneous anaesthesia over the region to be tested was produced by electrophoresis. A lint pad six by four cm, soaked in a solution containing 1 g of procaine and 0 003 g of adrenalin in 20 c c of water, was placed on the skin and a constant current of 10 milliamps passed for 15 minutes. By this method an area of cutaneous anaesthesia six by four cm can be produced which will last for one to two hours. If a needle is passed through this anaesthetic skin the normal deep pain and pressure spots (20) can be demonstrated in the deep fascia and tendon sheaths so that the anaesthesia is strictly cutaneous. We also tried producing cutaneous anaesthesia by blocking cutaneous nerves with procaine infiltrations, and in one of us (A J M) the dorsal cutaneous branch of the ulnar and the adjoining branch of the superficial radial nerves were exposed and crushed. This method was less satisfactory because, although it produced good cutaneous anaesthesia, exploration with a needle revealed extensive anaesthesia of the deep fascia and tendon sheaths on the dorsum of the hand. This was not surprising since Stopford (15) has shown that these cutaneous nerves frequently supply many of the deeper structures of the hand as well as the overlying skin.

Both deep pressure and deep pain sensibility were tested by applying graduated pressure to the part through a rounded cork or wooden applicator 1 cm in diameter. The pressure was applied by springs of different strengths so that we had four applicators. The lighter applicators with ranges of 0 to 100 g, 0 to 300 g, 0 to 800 g and 0 to 8 kg respectively. The lighter one for pressure sensibility and the heavier one for pain, for brevity the latter will be referred to as the algesiometer. In addition, deep pain sensibility was tested by exploring the tissues with a needle and by injecting 0 2 c c of 6% saline which is a very powerful pain stimulus. The deep pain response from cooling recently described by Wolf and Hardy (21), was also tested in some experiments by holding a block of ice over the part to be tested.

Cutaneous pain and touch sensibility were tested by the usual methods of pin prick and von Frey s hair, and by certain additional methods. The description by Bishop (3) of the

pricking pain unit of cutaneous sensibility made it desirable for us to know what was happening to " pricking " during our nerve blocks, and we also wished to use a graded mechanical stimulus which would be comparable to the stimuli used for testing deep sensibility. We, therefore, used a trocar pointed steel wire with a diameter of 0 5 mm , attached to a spring , by gradually increasing the pressure with which the pointed wire impinged on the skin, a gradation of sensations was produced. With light pressures, the wire only gave a sensation of contact, but with increasing pressure a sensation of pricking and finally pain became added. The pricking pain sequence was particularly clear when the wire was applied over one of the high spots of greater pain sensitivity described by Bishop (3), and we mapped out such spots on the hand and marked them for repeated testing

Touch sensibility was tested by stroking the skin with strips of paper 1 cm wide. These strips were cut from different weights of paper so that their bending strain, when applied at an angle of 45°, varied from 0 005 g to 5 0 g. Along the ulnar border of the hand the paper with a bending strain of 0 005 g could not be appreciated provided the hairy skin of the dorsum was avoided, the next weight 0·01 g was invariably just appreciated while the heavier papers gave a more pronounced sensation of touch. We have used this method of stroking because it brings out certain changes in touch sensibility which are not clearly appreciated if touch is tested by Frey's hair alone

RESULTS

Deep sensibility on the dorsum of the hand

After anæsthetising the skin over the dorsum of the hand by electro-phoresis, we investigated the remaining deep sensibility by means of the pressure applicators

A pressure of over 300 g always gave rise to a sensation of pressure The quality of this sensation was not unlike that of touch but it could be recognised as arising from the deeper structures. A continuous pressure of 300 g gave rise to a continuous sensation of pressure that remained constant for periods of over one minute, there being no adaptation , but with a pressure of 100 g , the sensation rapidly faded away so that after about 15 seconds it was no longer appreciated, and if the pressure was reduced slowly it could be removed altogether without arousing any further sensation If the pressure was reduced rapidly there was a slight but definite " off " sensation. With a pressure of 25 g , the adaptation was very rapid, the sensation fading away in less than a second. Because of this adaptation the threshold for deep pressure sensibility varied with the rate at which the pressure was applied. A pressure of less than 15 g was not appreciated at any speed of application. If the pressure was increased from 0 to 25 g in less than half a second a brief sensation like a tap was experienced, but if the pressure was increased at the rate of 10 g per second no sensation was experienced until the pressure reached well over 100 g. It is interesting to note that this adaptation of deep pressure parallels very closely the adaptation of paccinian corpuscles as described by Adrian (1). If the pressure was increased gradually above 300 g the sensation of pressure also increased gradually, and when the pressure reached one to three kg a sensation of deep pain became added to the sensation of pressure. With further increases of pressure the severity of the deep pain increased and its distribution became more widespread but there was no alteration in the quality of the pain. A similar result was obtained when the deep structures were explored with a needle point. When the needles impinged upon a sensitive spot there was an immediate sensation of pain. Increasing the

stimulus merely increased the severity of the pain but did not alter its quality   The threshold for deep pain as tested by the pressure algesiometer varied considerably from one part of the hand to another   We found the base of the fourth interosseous space the most satisfactory place for testing, and provided pressure was applied to the same spot and in the same direction successive readings varied by less than half a kilogram   The speed of application was also important   If the pressure was increased slowly over a period of more than 10 seconds the threshold readings remained constant, but with more rapid application there was a tendency to overshoot the end point and the threshold readings became much higher   We were unable to demonstrate any adaptation of pain   Thus on a spot where the threshold for pain was 1 kg this pressure was maintained for over five minutes without any significant alteration in the sensation of pain, and with greater pressures a similar result was obtained

It has been stated that cutaneous anæsthesia lowers the threshold of deep pain sensibility (7)   We were unable to confirm this   We applied graded pressures through both normal and anæsthetic skin, produced by electrophoresis, and found that deep pain was experienced with the same degree of pressure in either case , there was also no difference in the quality or the severity of the pain experienced   When cutaneous anæsthesia was produced by blocking or crushing the cutaneous nerves the threshold for both deep pain and pressure was markedly raised over the centre of the anæsthetic area   During recovery of such a nerve block and during regeneration of the crushed nerve, deep pain sensibility returned before cutaneous sensibility became normal and during this phase both excessive pain and a lowering of the pain threshold to pressure were noted, and we think this may account for the difference between our findings and those of previous observers (4, 7)   We noted, however, that cutaneous anæsthesia increased our awareness of deep sensations in the same way as one notices a faint noise more readily in darkness than in brilliantly illuminated surroundings

Deep sensibility in nerve blocks

Having become familiar with the normal deep sensibility in the back of the hand we proceeded to block the ulnar nerve at or above the elbow and to observe the failure and recovery of deep pain and pressure sensibility   Notes were also made of the onset of motor paralysis and of the behaviour of cutaneous pain and touch sensibility for comparison

The changes in deep sensibility which result from compression of the ulnar nerve by the clamp will be described first   During the first 15 to 20 minutes of compression no change could be detected in deep pressure sensibility , the threshold for quick application (less than ¼ second) remained at 25 g and the sensation produced by a continuous pressure of 300 g remained constant for a period of over 30 seconds   After about the 20th

minute the threshold for quick application began to rise and after 30 to 35 minutes of compression it rose to over 500 g and shortly after this deep pressure sensibility failed altogether, higher pressures giving rise to pain only As the threshold for quick application was rising the sensation produced by a continuous pressure of 300 g began to fade away so that when the threshold had risen to between 75 and 100 g the sensation from a continuous pressure of 300 g faded away completely in about 15 seconds, and when the threshold approached 300 g the sensation from a continuous pressure of 300 g faded in a second or two, thus a rising threshold was accompanied by more rapid and complete adaptation

Throughout this period deep pain sensibility altered little, though there was sometimes a slight lowering of threshold after 20 to 25 minutes of compression After 30 minutes of compression the threshold for deep pain, as tested by the algesiometer, began to rise slowly and after about one hour of compression the threshold rose to over 7 kg When this occurred no deep pain could be produced by a further increase of pressure, and no pain could be elicited from the deep structures by exploration with the needle point or by the injection of 6% saline Thus complete analgesia of the deep tissues resulted from about one hour of compression The deep pain response from local cooling failed more quickly so that no deep pain followed the application of ice when the algesiometer readings had risen to about 5 kg The immediate pain response from the skin failed completely before deep pain sensibility was materially reduced, while the skin continued to give a delayed pain response long after all deep pain sensibility was lost

When the clamp was released after one and a quarter hours of compression deep pain sensibility recovered rapidly, returning *to normal* within the first minute Deep pressure sensibility recovered more slowly during the second, third and fourth minutes after release and its recovery was accompanied by intense spontaneous sensations of "buzzing" and "cramp" felt in the ulnar part of the hand

Occasionally the ulnar nerve escaped proper compression and the resultant block failed to progress beyond a certain stage, but if care was taken to compress the nerve where it passes backwards just above the medial epicondyle, satisfactory blocks were obtained We have made a special study of deep pain sensibility in 20 satisfactory pressure blocks of the ulnar nerve In six of these blocks cutaneous anæsthesia of the dorsum of the hand was first induced by electrophoresis, while in the remainder deep pain was studied through normal skin We did not observe any difference in the behaviour of deep pain sensibility in these two series and we have, therefore, only used cutaneous anæsthesia in blocks in which we wished to study deep *pressure as well as* deep pain

In the ulnar nerve blocks produced by cooling the result was somewhat different While the temperature in the region of the nerve was falling from 30° to 15°C the subject experienced spontaneous deep pain felt in

the hand During this period the deep pain threshold for mechanical stimuli rose steadily and when the temperature in the region of the nerve reached about 10°C no pain could be elicited from the deep structures in the ulnar part of the hand by any form of stimulus

Deep pressure sensibility remained normal until the temperature of the nerve approached 5°C The subject then experienced a spontaneous sensation of tension and cramp in the hand, following this the threshold for pressure with quick application rose and adaptation to a continuous pressure of 300 g became more rapid and complete In our cold blocks deep pain sensibility was lost when the temperature in the region of the nerve approached 10°C At a slightly lower temperature the immediate skin pain response began to fail, while the delayed skin pain response remained unaffected by much lower temperatures There was thus a clear dissociation of deep and cutaneous pain

When cooling was stopped deep pressure sensibility returned rapidly to normal and as the nerve warmed up deep pain sensibility also returned, and during this period some spontaneous pain was occasionally felt in the hand, though this was much less than during cooling

During procaine blocks function usually failed rapidly but with a perineural infiltration the onset of paralysis was sometimes spread over as long as 30 minutes In such a block deep pain sensibility failed during the first 10 to 15 minutes, while deep pressure sensibility persisted till the end, its failure following the familiar pattern of a rising threshold accompanied by more rapid and complete adaptation During recovery deep pressure sensibility returned early, while deep pain sensibility recovered much later During the onset of procaine blocks, we were unable to make out any constant dissociation between deep pain and the immediate and delayed skin pain responses, all three seem to fail about the same time in rather a patchy manner, but during recovery from a prolonged procaine block the delayed skin pain response returned first, deep pain recovered shortly after this, while the immediate skin pain response returned somewhat later But there was no clear dissociation between deep and cutaneous pain in these blocks

During the nerve blocks produced by all three methods, we found that while the threshold for deep pain was rising, the threshold pressure occasionally gave rise to pain of greater severity than that produced by the same pressure applied to the normal hand The pain also did not increase gradually as the pressure was increased but became immediately severe and tended to last longer so that the pain response had an excessive and explosive character There was, however, no alteration in the quality of the pain and to a suitable stimulus the response remained immediate throughout, there being no period of delayed deep pain

With our methods of testing deep pressure sensibility we failed to elicit a similar explosive response, and there was certainly no alteration in the quality of the sensation of pressure In all the blocks the failure of pressure

sensibility following the same pattern of rising threshold accompanied by more rapid and complete adaptation, the only other abnormality being the spontaneous sensation experienced during cooling and after release of the clamp

Vibration sense was tested in all these blocks by a tuning fork applied over the head of the fifth metacarpal The sensation of vibration became markedly reduced when pressure sensibility failed, but it was never altogether abolished, presumably because some vibration was transmitted to the non-anæsthetic part of the hand

Cutaneous sensibility in nerve blocks

The behaviour of cutaneous sensibility in nerve blocks has already been thoroughly investigated, but by the use of the wire stimulator and the graded papers we have noted some changes in both pain and touch sensibility which we think are worth recording

Pain When the end of the wire stimulator was held lightly upon normal skin a sensation of contact was experienced If the wire was applied with increasing pressure a sensation of pricking became added at a pressure of 50 to 70 g (By pricking we mean a punctate sensation with the quality of pain but without its unpleasant affective character) With a pressure of 90 to 110 g , the subject experienced definite pain , if the pressure was further increased gradually the pain increased in severity The transition through pricking to pain was a gradual one and it was not easy to decide the exact moment at which pricking was first felt, or when pricking gave place to pain so that there was a good deal of scatter between successive readings, the thresholds for pricking and pain varying by as much as 20 g

During the onset of a pressure block no change could be detected in the sensations produced by the wire until about the 20th minute of compression Then the threshold for pain began to fall so that after about 25 minutes of compression the wire gave rise to pain at a pressure of about 50 g At this stage there was no preceding sensation of pricking, and the pain was at once severe and persisted for some time after the stimulus was removed The end point was now very clear and successive readings for the pain threshold were consistent to within 5 g At this stage a needle prick still gave rise to immediate pain With further compression the wire continued to give rise to severe pain at about 50 g , but the quality of the sensation changed, the pain acquiring an itchy character which is usually described by the word stinging, and the pain following each stimulus gave place to itching as it faded away At this stage a needle prick gave delayed pain only Nerve compression has been continued for over one and a quarter hours, but although the threshold for stinging delayed pain tended to rise towards the end of this time, we have been unable to abolish this pain

response altogether   In these blocks two changes were noted in pain sensibility   First the gradual transition through pricking to pain was replaced by an explosive pain response at the original threshold for pricking, and at a later stage there was an alteration of quality to stinging, the stinging pain being always delayed   When the clamp was released pain sensibility recovered rapidly, the normal pricking-pain gradation returning within the first minute

In procaine blocks pain sensibility failed more rapidly and the sequence of events was less clear, but with blocks of slow onset there was a similar period of explosive pain response   During recovery, however, a similar sequence of events occurred   The delayed stinging pain returned first and passed through a period of low threshold and explosive response , then the pain response to a needle prick became immediate and the quality of the pain returned to normal, but the threshold remained low and the response remained explosive and excessive and only gradually gave place to the normal pricking pain gradation

In the blocks produced by cooling the results were similar to those observed in pressure blocks   As the temperature of the nerve trunk dropped, the pricking pain gradation gave place to an explosive pain response and when the temperature in the region of the nerve sank to below 8°C the quality of the pain changed to stinging and the response to pin prick became delayed only   As the nerve warmed up the same changes took place in reverse order

Touch   In some of our nerve blocks we noticed that touch sensibility also passed through an abnormal phase in which stroking the skin gave rise to a curious excessive buzzing sensation which resembled exactly the sensation produced by touching the arm of a vibrating tuning fork   This buzzing response was well displayed by stroking the skin with the graded strips of paper

During the onset of an ulnar block produced by a pressure of 70 mm Hg no change in touch sensibility occurred during the first 15 minutes of compression   Then the papers began to give rise to the buzzing sensation and for a minute or two the lightest paper which was normally not appreciated was felt quite distinctly   Then the threshold began to rise and the lighter papers failed to give rise to any sensation, while the heavier papers continued to give a buzzing response until the 25th to 30th minute of compression when touch sensibility was lost altogether   When the clamp was released, touch sensibility returned during a period of intense buzzing   In procaine blocks the same buzzing response occurred while the threshold for touch was rising, and during recovery the buzzing response recurred while touch sensibility was returning to normal   In nerve blocks produced by cooling touch sensibility also passed through a similar buzzy phase which was also accompanied by a spontaneous sensation of buzzing felt in the ulnar territory of the hand

The buzzing phase of touch sensibility and the explosive phase of pain sensibility were both more pronounced in some nerve blocks than in others Thus in procaine blocks these abnormal phases were only pronounced when the onset of the block was gradual, and when recovery was spread over a prolonged period such as one hour or more   These abnormal sensations were also more evident on the palmar aspect of the hand than on the dorsum, and in blocks of the small cutaneous nerves of the forearm and leg sensation simply failed without any preceding abnormal phase   In pressure blocks produced by the clamp the buzzy phase of touch sensibility was only pronounced when the pressures used were near the threshold at which paralysis occurred, that is between 60 to 70 millimetres of mercury , and in such blocks the abnormalities of pain sensibility were also more pronounced than they were when the nerve was compressed by higher pressures   In cold blocks the abnormalities of pain and touch sensibility were more pronounced with rapid cooling than with slow cooling

DISCUSSION

Although the changes occurring in deep and cutaneous sensibility have been described separately, in most of our nerve blocks they were studied simultaneously in order to establish the position of deep pain and deep pressure in the general scale of function dissociation   Our results in relation to those of previous observers are shown in the table

TABLE I.

Shows the order of fibre paralysis in nerve blocks   The left hand columns represent the results of previous observers   Bickford (2), Lewis and Pochin (14) and Heinbecker, Bishop and O'Leary (8)   The right hand columns show the relative positions of deep pain and pressure as determined in our own blocks

Cold Block		Pressure Block		Procaine Block	
Cold		Touch	`	Vasomotor	
	Deep pain	Pressure	Pressure	Cold	
Motor		Motor		Warmth	
Vasomotor		Cold		Skin pain }	
1st pain		1st pain		Deep pain }	Deep pain
Touch		Warmth		Motor	
	Pressure		Deep pain	Pressure }	
2nd pain		Deep pain }		Touch }	Pressure
Warmth		2nd pain }			
		Vasomotor			

As previous observers have noted (8, 14) deep pressure fails about the same time as touch and is widely dissociated from deep pain   In procaine blocks deep pain fails about the same time as cutaneous pain, but in blocks produced by cooling deep pain fails very early and is widely dissociated from cutaneous pain which fails late   In blocks produced by pressure deep pain persists after the immediate skin pain response has been abolished, but fails

altogether before the delayed skin pain response is materially reduced. It is interesting to note that the failure of deep pain is not preceded by any alteration in quality, and that the distinction between the quality of deep and cutaneous pain is never lost

The clear dissociation which occurs between deep and cutaneous pain in cold blocks and the constant dissociation between deep pain and pressure gives further support to the view that deep pain should be considered as a separate mode of sensibility, and the fact that the deep pain response is always immediate makes it unlikely that the impulses subserving deep pain are transmitted by slow conducting fibres of the C type

Cutaneous hyperalgesia has been previously noted as occurring in nerve blocks produced by pressure (14) and cooling (2, 18), but from our observations it appears that under certain circumstances touch sensibility, as well as pain sensibility, may pass through a similar abnormal phase in which the sensory response to mechanical stimuli is both excessive and explosive in character   This abnormal phase seems to occur during the period of failure and during recovery of that particular mode of sensibility and does not appear to be dependent upon the withdrawal of the other   Thus in procaine and cold blocks pain sensibility passes through the abnormal phase while touch is still normal, while in pressure blocks touch passes through the abnormal phase before pain is materially affected   If we accept the views of Zotterman (22) and Lewis (13) that there are two forms of cutaneous pain— the immediate pricking pain subserved by fast conducting fibres and the delayed stinging pain subserved by slow conductors—then the complicated changes in skin pain sensibility which occur in nerve blocks may merely represent the successive abnormal phases of the immediate (pricking pain) and the delayed (stinging pain)

SUMMARY AND CONCLUSIONS

1   Both deep and cutaneous sensibility have been studied in blocks of the ulnar nerve produced by pressure, cooling and procaine infiltration

2   In nerve blocks produced by pressure and cooling deep pain sensibility was dissociated from both cutaneous pain and deep pressure During the failure of deep pain sensibility there was no alteration in the quality of the pain and no delay in its onset   These findings give further support to the views that deep pain should be considered as a separate mode of sensibility and that deep pain is not carried by slow conducting fibres of the C type

3   Under certain circumstances both touch and cutaneous pain sensibility may pass through an abnormal phase which is characterised by an excessive and explosive sensory response to mechanical stimuli   This abnormal phase occurs during the period of failure and during recovery of that particular mode of sensibility

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ON DEEP HYPERALGESIA AND COLD PAIN

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INTRODUCTION

AMONG the many patients attending the Wingfield-Morris Hospital for painful conditions of the extremities we found a number in whom spontaneous pain was only experienced when the affected part was cool and in whom warming the part gave complete relief   This phenomenon of " cold pain " was observed in patients suffering from painful states following a variety of traumata, such as simple fractures, gunshot wounds of peripheral nerves and minor digital nerve injuries

In the patients who suffered from cold pain the affected extremity was often cooler and more cyanosed than the normal and interruption of the sympathetic supply to the part resulted in a varying degree of relief from pain   Because of these features we were tempted to consider these cases as examples of painful vasomotor disorders (3, 5, 6, 9, 15) or minor causalgia (4, 10, 15, 16), but the relation of pain to cooling and vascular disturbance was far from clear, and the results of sympathectomy were by no means uniformly successful

Spontaneous pain is felt in the normal hand when the ulnar nerve is cooled rapidly (1)   We find that this pain is deep and is experienced while the temperature of the nerve is falling from 30° to 15°C   As the temperature of the hands and feet frequently falls within this range during cold weather it seemed likely that the cold pain of which our patients complained might be caused by an abnormality of deep pain sensibility, and we have carried out both clinical and experimental investigations to explore this possibility

CLINICAL INVESTIGATION.

Method.

The first step was to determine the quality of the patient Skin pain is conveniently produced by pulling upon a few hairs on the dorsum of the hand, while squeezing the muscles of the first interosseous space produces a satisfactory deep pain Most patients had no difficulty in recognising the difference between these two sensations, and in deciding which of them resembled their spontaneous pain In all cases included in this investigation the pain induced by cold was deep, having the same quality as pain arising from the muscles and joints, and throughout this investigation we have been dealing with deep pain as distinct from skin pain

The following test was used to determine the presence or absence of abnormal pain from cooling Both normal and abnormal extremities were warmed to 37°C in a water bath Both extremities were then plunged into water at 20°C and kept there for five minutes If no pain was experienced the procedure was repeated with water at 15°C At the lower temperature slight pain was usually experienced in the normal limb for a minute or two If cold pain was pronounced the abnormal limb gave rise to pain with water at 20°C and to severe pain at 15°C , when cold pain was less pronounced it was only experienced at 15°C, but the pain was more severe and more prolonged than on the normal side If temperatures as low as 10°C were used severe deep pain was experienced in the normal hand, and with still lower temperatures the deep pain was accompanied by skin pain and tingling If the test was repeated with the circulation occluded by a cuff on the arm or leg, the result was essentially the same , the warm water relieved pain while the cold caused the same pain as previously, though its onset was a little more rapid But in a few patients in whom the normal vasoconstriction which accompanies cooling had been interfered with by a recent sympathectomy, pain was only experienced when the test was carried out with the circulation occluded

The deep pain threshold for mechanical stimuli was determined by a spring algesiometer with a rounded wooden applicator one centimetre in diameter By this apparatus graded pressures were applied to the most sensitive part of the abnormal extremity, and to the corresponding part of the opposite normal extremity The pressure in kilograms at which deep pain was first experienced was noted The pressure was then increased by a further ½ kg and the severity, duration and distribution of the resultant pain from the two extremities was compared

For assessing the peripheral vascular disturbance in these patients we have used oscillometry, reactive hyperæmia (13) and records of the skin temperatures

Clinical cases

Major nerve injuries The following two case histories are typical examples of peripheral nerve injuries in which " cold pain " was a prominent symptom

Case I

Lieutenant J R.G   Aged 25 years   A farmer in civilian life

History   On 23 12 43, while serving in Burma, he received multiple wounds of the right leg complicated by an incomplete paralysis of the sciatic nerve   When first seen on 5 9 46 the sciatic paralysis had largely recovered but he suffered from continuous severe pain in cold weather which prevented him from farming and kept him indoors with his foot on the stove

Progress of nerve injury by 5 9 46   Motor recovery was good   All the muscles of the leg except the intrinsics of the foot, were acting at (4) to (5) (M R C grading)   Sensory recovery was fair   Three areas of cutaneous anæsthesia and analgesia remained   one over the front of the ankle, another under the lateral metatarsal heads and a third on the plantar aspect of the heel   On the remainder of the sole cutaneous pain sensibility was normal but there was some non painful hyperæsthesia

Pain   The pain was deep and was felt diffusely in the foot, but when severe it spread up the leg to the knee   The pain had been present since wounding and, although it had improved during the first year, it was now stationary   Spontaneous pain came on whenever the foot became cold and complete relief was obtained while in a hot bath

Cold test

Temperature of water	Right foot	Left foot
20°C	Deep pain in foot	No pain
15°C	Severe pain spreading up the leg	No pain

Algesiometer

Sole of foot		
	0 6 kg, pain	1 4 kg, slight pain
	1 1 kg, severe pain spreading up the leg	1 9 kg, slight pain in foot only

Vascular

Skin temperatures oscillometry and reactive hyperæmia revealed no significant difference between the right and left legs

On 11 9 46 a right lumbar sympathectomy was performed   the lumbar chain L 2 to L 4 being removed   This resulted in relief of cold pain

On 15 10 46 the tests were repeated   The cold test was now negative, no pain being experienced in water at 15°C, but the algesiometer still gave an excessive pain response in the right foot and the deep pain threshold to pressure and the abnormalities of cutaneous sensibility remained unchanged   The vascular condition was altered in that the skin temperature of the right foot remained 6° to 9°C higher than the left after exposure to a room temperature of 16°C   If the circulation to the right foot was occluded by a cuff on the thigh, the cold test became positive again

Since the operation he has returned to farming and has only experienced a little pain during exceptionally cold weather

Case II

Rifleman G C   Aged 22 years   A shoemaker in civilian life

History   On 4 9 44 while serving in Belgium he received bullet wounds in the upper third of the left arm   The brachial artery and median nerve were completely divided and the ulnar nerve was partly divided   On 4 12 44 both median and ulnar nerves were resected and sutured

Progress of nerve injury by 20 2 47   Motor recovery fair and still progressing   Forearm muscles all acting at (4) to (5)   Intrinsics of the hand at (2) to (3)   Sensory recovery fair and still progressing   No loss to light touch or pin prick but discrimination poor

The hand was useful but he suffered from severe pain in cold weather which prevented him from working and he also suffered from slight intermittent claudication of the forearm muscles

Pain   The pain was deep and felt in the palm of the hand and " across the knuckles "; when severe it spread up the forearm   The pain was first experienced one year after the nerve suture and was severe whenever the hand became cold

Cold test

Temperature of water	Left hand	Right hand
20°C	Slight ache	No pain
15°C	Severe pain	No pain

Algesiometer

Dorsum of hand	1 5 kg , pain	2 5 kg., slight pain
	2 0 kg , pain	3 0 kg , pain

Vascular

On exposure the left hand became more cyanosed and about 5°C cooler than the right The oscillations were greatly reduced in the left forearm and the reactive hyperæmia was delayed, the flush taking over 10 seconds to appear in the hand   A left dorsal sympathetic block caused a rise in the temperature of the hand of 10°C , accompanied by relief of pain

On 24 2 47 a left dorsal sympathectomy was performed (White's technique) (14)   Following this the left hand became warm and the cold pain was relieved

On 5 3 47 the tests were repeated   The cold test was negative in that no pain was experienced with water at 15°C   The algesiometer readings remained unchanged, 1 5 kg still giving excessive pain in the left hand   The vascular condition was much improved, the skin temperature of the left hand remaining 2° to 3°C higher than the right after exposure to a room temperature of 14°C   The oscillations of the left forearm were also improved, though still less than those of the right forearm

On reviewing 50 cases in which a major nerve injury was associated with pain we found a further 13 patients in whom cold pain was a prominent symptom   In all 15 cases deep hyperalgesia to mechanical stimuli was marked, and in many of the cases there was also a disturbance of cutaneous sensibility in that stimuli applied to the skin gave rise to abnormal and exaggerated sensations   Of these 15 cases three were plexus lesions and six were partial lesions of major nerves with incomplete recovery   In the remaining six one or more nerves had been resected and sutured   In the sutured cases the pain was experienced during the period of regeneration

The vascular condition of the limb in these 15 patients differed considerably   In two patients the main artery had been tied and the peripheral circulation was deficient   In a further six patients the affected extremity was colder and more cyanosed than the normal but there appeared to be no organic vascular defect   While in the remaining seven patients the peripheral circulation appeared to be normal

Among the 15 patients suffering from cold pain six had sympathectomies In these six patients cold pain was greatly improved in four but only slightly improved in the remaining two   In the unsatisfactory cases there was no marked temperature rise in the extremity following the operation   In all six cases the deep hyperalgesia to mechanical stimuli remained unchanged, and the abnormalities of cutaneous sensibility persisted so that it was only the pain from cooling that was affected by sympathectomy

Digital neuromata   The following case is a typical example of a digital neuroma with cold pain

W B   An engineer aged 52 years

In 1943 he crushed the tip of his left fifth digit   The wound healed normally but during the following year he developed a sensitive spot in the pulp of the finger which became progressively more sensitive in spite of three operations for resection of the digital nerves   If the finger became cold he suffered from continuous pain but warming the hand gave complete relief

When first seen on 1 10 46, the finger did not appear grossly abnormal   A small scar from the original injury could be seen on the ulnar side of the finger just beyond the distal interphalangeal joint   At the base of the finger and in the palm were the scars of previous neurectomies   The scars were well healed and not hypersensitive   In the pulp of the finger on its ulnar side, about 1 cm distal to the scar, there was an acutely sensitive spot about 2 mm in diameter   There was cutaneous analgesia and anæsthesia of both palmar and dorsal aspects of the ulnar half of the finger   The tender spot lay just within the area of cutaneous sensory loss

Pain   The pain was deep, felt maximally in the finger but spreading up the inner side of the arm to the axilla when severe

Cold test

Temperature of water	Left hand	Right hand
20°C	Severe pain	No pain
Repeated with circulation occluded		
27°C	Pain	No pain
28°C	No pain	
27°C	Pain.	
28°C	No pain	

Algesiometer

5th digit over tender spot	Less than 10 g Pain severe and spreading up the arm.	1 0 kg Slight pain

Vascular

No definite abnormality

9 10 46   Local excision of a small pearly grey nodule 2 mm in diameter from the subcutaneous tissue of the finger   This gave complete relief, and when seen nine months after the operation there had been no recurrence of pain or tenderness

On reviewing 32 cases of painful digital nerve injuries we found a further nine cases who presented some degree of cold pain, together with proximal reference of pain up the arm   Cold pain was also a prominent feature in a case of glomus tumour of the foot

In most of these cases the painful extremity was somewhat cooler and more cyanosed than the normal, and in one case a great delay in reactive hyperæmia, suggested organic obstruction of the digital vessels in the affected finger   This case was the only one in which sympathectomy was performed   While in hospital this patient was relieved of pain but on returning to his outdoor occupation the finger became blue and cold again and the pain returned, so that the finger was finally amputated

Simple fractures   By arrangement with Mr Scott of the Accident Service of the Radcliffe Infirmary, we were able to investigate a number of patients who suffered from painful extremities following a simple fracture A few of these patients suffered from marked cold pain   The following case is a typical example

Mrs W S   A housewife aged 50 years

On 19 12 46 she fell and fractured her right forearm   X ray revealed a Colles fracture with much comminution of the joint surfaces   The fracture was reduced and maintained in

plaster for five weeks   While in plaster she was fairly comfortable but after removal of the plaster she suffered much pain whenever the wrist became cold, and the pain was also aggravated by movement of the wrist and use of the hand   Warming the wrist gave complete relief

When examined on 22 3 47 there was some swelling over the wrist joint and movements were restricted   flexion 0° to 60°, extension 0° to 30°, pronation and supination nearly full   Movements of elbow and shoulder were full and painless

Pain   Deep in quality, felt mostly in the wrist but with some spread up the arm to the shoulder

	Cold test	
Temperature of water	*Right wrist*	*Left wrist*
20°C	Pain	No pain
15°C	Severe pain up to shoulder	No pain
	Algesiometer	
Over wrist joint	2 0 kg , pain	4 5 kg , slight pain
	Vascular	
	No definite abnormality	

This patient was treated by physiotherapy and re-education and two months later she was nearly free from pain and the range of wrist movements had improved

Out of ten patients with Colles fracture we found a further two with similar cold pain   All three patients with cold pain had unusually severe fractures resulting in considerable disability during convalescence, but the fractures were not complicated by injury to the main vessels or nerves of the limb

Discussion of clinical cases

The patients complaining of cold pain all presented certain common features   Firstly, the pain had the same quality as pain arising from the muscles and joints, and had the same diffuse distribution with a tendency to proximal reference from the extremity up the limb   Secondly, the deep tissues at the source of pain were abnormally sensitive to mechanical stimuli , pressure giving rise to more severe pain and at a lower threshold than normal   This clinical picture of deep hyperalgesia with cold pain appears to be sufficiently striking to warrant its consideration as a definite clinical entity, especially as it seems likely that a common pain mechanism is responsible for this symptom in the various patients in whom it occurs The role of vascular disturbances in this pain mechanism is, however, far from clear

Although there was a definite vascular disturbance in some of our cases, the circulation appeared to be normal in many of those patients who suffered from the most severe cold pain, and we observed many patients with severe vascular disturbances who did not experience excessive pain on cooling   Although all the patients with cold pain had some degree of deep hyperalgesia as shown by the algesiometer, some cases with quite marked deep hyperalgesia did not suffer from cold pain   At first we thought cold pain might only occur in patients with lesions of the peripheral nerves but we soon found that it occurred in patients with simple fractures and other

conditions causing deep hyperalgesia of the extremities   Thus the only
constant finding in our patients suffering from cold pain was deep hyperalgesia
of the affected part, and it seemed to us that their spontaneous pain was
most probably due to an abnormal sensitivity of the deep pain nerves to
the stimulus of cold   Before further investigating this possibility it was
clearly necessary to know more about the effect of cooling on deep pain
sensibility in normal subjects   We, therefore, carried out the following
experiments

EXPERIMENTAL INVESTIGATIONS

Method   The experiments about to be described were carried out
upon ourselves   Each observation was thus confirmed by three different
individuals, and the observations were repeated several times on each
individual on different days   The three subjects were familiar with the
differing quality of skin pain and deep pain and all remarks in these
experiments refer to deep pain unless otherwise stated   Spontaneous pain
is a purely subjective phenomenon so that we have simply noted its presence
or absence and tried to assess its severity by an arbitrary scale of one to five
units, five units being the most severe pain we were prepared to experience

Deep pain sensibility was tested by the algesiometer which we had
used in the clinical investigation, and in our studies of deep pain sensibility
in nerve blocks   The presence of analgesia was confirmed by exploring the
deep tissues with the needle point and by injecting 0 2 c c of 6% saline
The skin temperature was recorded by a thermocouple attached to the skin
with adhesive tape, and the temperature of the deep structures was recorded
by thermocouples cemented into intramuscular needles by varnish   The
thermocouple junction occupied the terminal 1 cm of each needle, and the
needles were so arranged that the thermocouple lay parallel to the surface
of the limb at the desired depth

If the whole hand or foot was to be cooled, the limb was immersed in a
water bath at the desired temperature, but if only a small area was to be
cooled a block of ice was used   The surface of ice applied to the limb being
5 cm square   The rate of cooling was adjusted by covering the ice with
one or more layers of lint

It was recognised from the outset that very accurate correlations
between temperature and deep sensibility would not be possible because of
the temperature gradient between the skin and the centre of a limb, and
because of the impossibility of distinguishing between pain arising from
different deep structures and therefore of knowing at what depth the pain
arises   In spite of these obvious limitations the method has proved adequate
for our purpose

Behaviour of deep temperatures on cooling   The deep temperatures have
been recorded in the hand, the arm and the leg   One thermocouple was

placed on the deep fascia and one in the underlying muscle about 1 cm deep to the fascia, a third thermocouple was attached to the skin Fig 1 illustrates the behaviour of the deep temperatures when a block of ice was placed on the skin of the leg   Subject 1 (J H K) cooled through more rapidly and more completely than subjects 2 (A J M) or 3 (E S H) Subject 1 also experienced more pain during cooling   This difference was noted throughout our observations, and was probably due to the absence
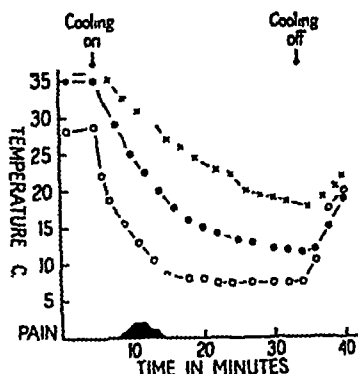
Fig 1   Illustrates the result of cooling the tibialis anticus in subject 2 (A J M) by a block of ice 5 cm square wrapped in lint and placed on the overlying skin   The temperatures were recorded by three thermocouples , one on the skin (circles), one on the deep fascia (dots), and one lying in the muscle 1 cm deep to the fascia (crosses)   Spontaneous deep pain is represented by the black areas, its severity being recorded on an arbitrary scale of one to five units , time is in minutes in this and all subsequent figures

of ‘subcutaneous fat in subject 1   As might be expected the deep temperatures alter more slowly than the skin temperatures and remain from 1° to 20°C higher   After about 30 minutes of cooling a state of equilibrium is reached and the temperatures remain fairly constant for that degree of cooling

The effect of cooling upon deep pain sensibility   If the warm hand is plunged into cold water (10°C) the subject first experiences a sensation of cold, this quickly fades away and is replaced by a sensation of deep pain which increases during the first two minutes and then fades away so that after four or five minutes the hand feels quite comfortable, neither cold nor pain being appreciated   If lower temperatures are used (5°–0°C) the deep pain is followed by a similar wave of skin pain and tingling

This phenomenon was investigated by Wolf and Hardy in 1941 (18) They attributed the fading of the pain to "adaptation" and suggested that the pain was "vascular" in origin   We have repeated their observations and agree in general with their findings, but our additional observations on deep pain sensibility suggest a different explanation for the fading away of the pain. If the hand is cooled by lowering the temperature of the water

bath in stages of 5°C a wave of pain follows each drop in temperature (Fig 2) If at the same time deep pain sensibility is tested with the algesiometer we find that the threshold for deep pain rises as the temperature
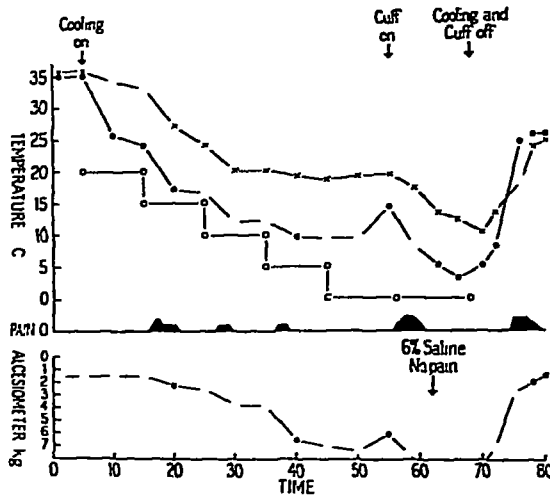
Fig 2 Illustrates the result of cooling the hand of Subject 2 in a water bath. Temperatures recorded by three thermocouples, one in the water bath (circles), one on the deep fascia of the dorsum of the hand (dots), and one placed deeply between the adductor pollicis and the first dorsal interosseous muscle (crosses) Spontaneous deep pain is represented by the black areas When the water temperature was 5°C or lower, considerable skin pain was also experienced in the hand For simplicity this has been omitted from the figure At 55 minutes the circulation to the hand was occluded by inflating a cuff on the upper arm
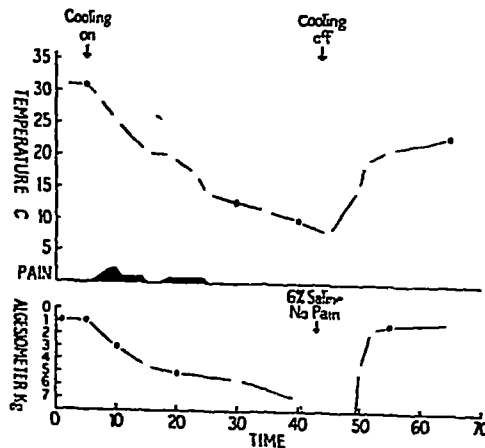
Fig 3 Illustrates the result of cooling the subcutaneous surface of the tibia in Subject 1 by means of a block of ice placed on the overlying skin The temperatures were recorded by a thermocouple placed on the periosteum Deep pain sensibility was tested by the algesiometer applied to the centre of the cooled area

falls, and when the temperature in the centre of the hand reaches about 10°C deep analgesia becomes complete   The conditions in the hand are, however, very complex because the algesiometer stimulates a considerable depth of tissue and because cold vasodilation (8) interferes with progressive deep cooling at the lower temperatures

We, therefore, carried out similar observations on the subcutaneous surface of the tibia where a thin layer of sensitive periosteum is supported by insensitive compact bone and where vascular reflexes are less evident, (Fig 3)

An area of leg 5 cm square is cooled by a block of ice   While the temperature of the underlying periosteum is falling from 30°C to 15°C the subject experiences spontaneous deep pain   During this period the mechanical threshold for deep pain rises steadily, and when the temperature of the periosteum sinks to about 10°C no pain can be elicited from this membrane by any form of stimulus
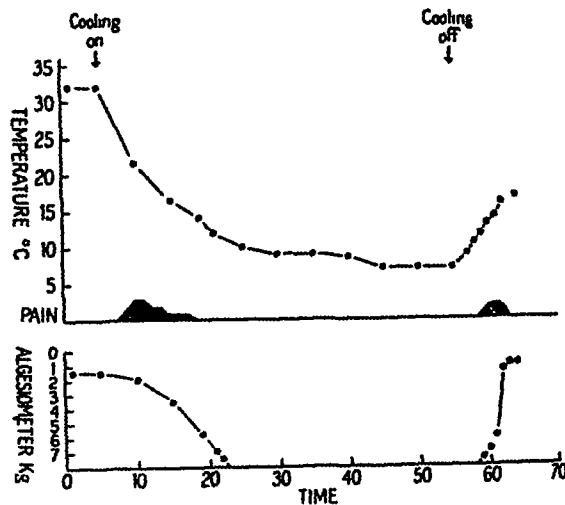
Fig 4   Illustrates the result in Subject 1 of cooling the ulnar nerve by immersing the elbow in a bowl of ice and water   The temperature is recorded by a thermocouple placed on the deep fascia just proximal to the ulnar groove   Spontaneous pain in the hand is shown in black   Deep pain sensibility was tested on the dorsum of the hand over the fourth interosseous space

A similar result is obtained from cooling a large nerve trunk   Fig 4 illustrates the result of cooling the ulnar nerve at the elbow   Again spontaneous deep pain is experienced in the hand while the temperature in the neighbourhood of the nerve is falling from 30°C to 15°C, and when the deep temperature at the elbow sinks to about 10°C no pain can be elicited from the deep structures in the ulnar border of the hand

In all our experiments spontaneous pain was most severe with rapid cooling, while with very slow cooling deep analgesia may develop with

little, if any, preceding pain   In this respect our findings agree with those of both Bickford (1) and Wolf and Hardy (18)   We have also noted occasional pain during warming up from 15°C to 30°C

From these observations we concluded that in normal subjects cooling the deep tissues rapidly through the temperature range 30°C to 15°C causes spontaneous deep pain   During the process of cooling analgesia of the deep tissues develops so that pain is only experienced for a short time while the temperature is actually falling   Although analgesia clearly results from the low temperature of the tissues, it is not clear whether the stimulus for pain is the low temperature as such or the temperature gradient during cooling and to a less extent during warming up   In the hand cold vasodilatation prevents progressive cooling so that the spontaneous pain may fade away before the development of complete analgesia

Effect of cooling upon experimental deep hyperalgesia   The subcutaneous surface of the tibia is clearly a most suitable part in which to investigate these phenomena   We, therefore, searched for a method of producing hyperalgesia of the periosteum in ourselves   After trying various methods, both mechanical and chemical, we found a suitable agent in calcium chloride

If an aqueous solution of 2% calcium chloride is injected into the periosteum in amounts such as 0 3 c c , there is little or no pain immediately after the injection, but an hour or two later deep tenderness develops at the site of the injection, so that the deep pain threshold of the periosteum becomes markedly lowered over an area about 2 cm square   Within this area the algesiometer readings become reduced from the normal, 1 to 2 kg to 0 2 to 0 4 kg   This deep hyperalgesia lasts from 24 to 48 hours   Provided the part is kept warm and at rest no spontaneous pain is experienced   By using this method we were able to compare the effects of cooling upon both normal and abnormally sensitive periosteum

The tender area of periosteum was mapped out and the algesiometer readings were noted   The corresponding area was marked out on the opposite leg to act as a control   A deep thermocouple was inserted so as to lie upon the periosteum just to one side of the tender area and the part was then cooled with a block of ice   The temperature of the periosteum was read at frequent intervals while the subject made a note of the presence or absence of pain and assessed its severity   Deep pain sensibility was also tested from time to time by the algesiometer   The whole process was then repeated over the control area in the normal leg   These observations were repeated with different degrees of hyperalgesia and rates of cooling   Most of our results varied between the two examples shown in Fig 5, but a few observations were made with very rapid cooling to below 10°C within 5 minutes   Although we were unable to produce exactly comparable cooling curves, we think certain general conclusions are warranted   With rapid cooling to low temperatures the difference between the two legs was not

very great (Fig 5A)   Although the hyperalgesic periosteum gave rise to more severe pain during cooling, the pain started at about the same
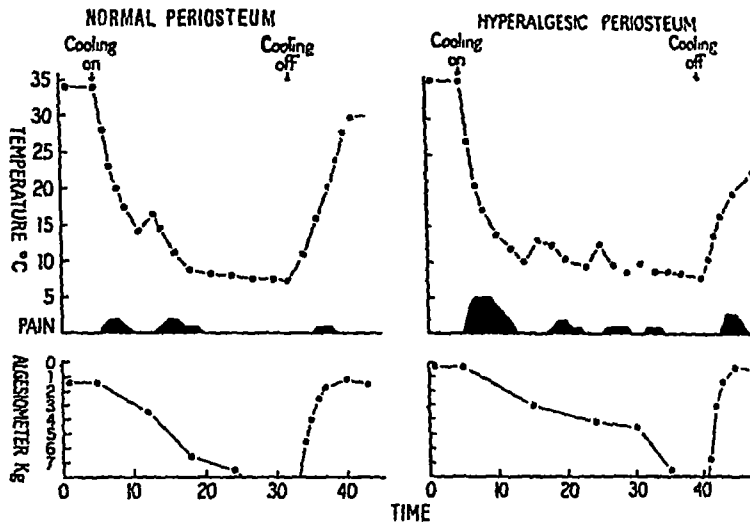
Fig A
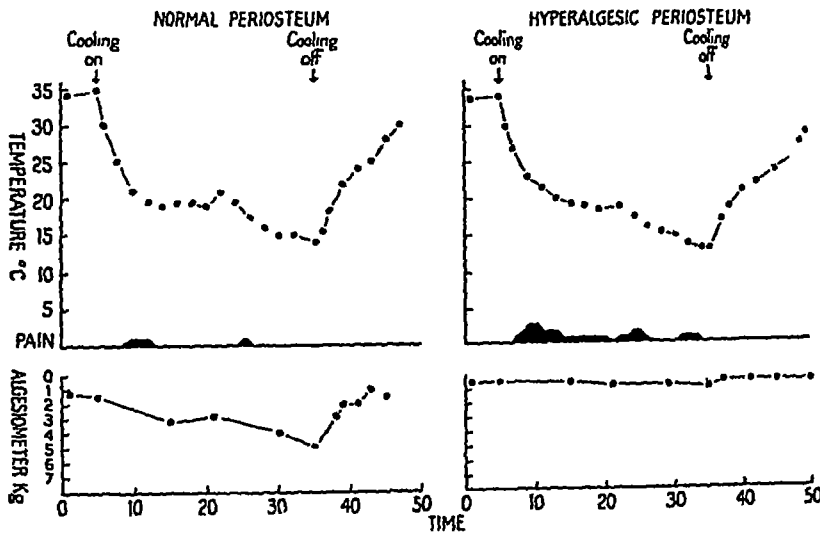
Fig B

Fig 5   Illustrates the result of comparable cooling of normal periosteum and periosteum that had been rendered hyperalgesic by the injection of 0 3 c c calcium chloride two hours previously   As before, temperatures were recorded by a thermocouple placed on the periosteum and deep pain sensibility was tested by the algesiometer applied to the centre of the cooled area   Fig A shows the result of rapid cooling to low temperatures in Subject 1   Fig B shows the result of slower cooling to intermediate temperatures in Subject 2

temperature and analgesia became complete at about the same temperature in both legs   With slower rates of cooling to intermediate temperatures, the difference between the two legs was more striking   (Fig 5B)   The

hyperalgesic periosteum gave rise to pain which started at a higher temperature and was both more severe and more prolonged than on the normal side, and the partial analgesia which developed in the normal periosteum developed imperfectly or not at all in the hyperalgesic periosteum

To sum up, we may say that slow cooling of a normal extremity from 30° to 15°C causes partial deep analgesia but no pain, but when deep hyperalgesia is present the same degree and rate of cooling causes severe and prolonged pain, but little or no analgesia

DISCUSSION

The effect of cooling upon deep pain sensibility is clearly very complex When the normal deep tissues are cooled rapidly, spontaneous pain is experienced for a short time while the temperature is actually falling At the same time analgesia develops so that in spite of continued cooling the spontaneous pain fades away With slow cooling analgesia develops without appreciable spontaneous pain

When deep hyperalgesia is present, even slow cooling of the affected part causes severe and prolonged pain, and the analgesia which normally accompanies cooling develops imperfectly unless the tissues are cooled to low temperatures (about 10°C)

We would like to suggest that a disturbance of this nature is responsible for the cold pain experienced by patients who have deep hyperalgesia of the extremities, and that in future it would be useful to investigate clinical cases of cold pain along these lines

If, on the assumption that this is correct, we reconsider our clinical cases some of their most puzzling features become clear Thus clinical cold pain probably results from the interaction of two factors Firstly, the degree of hyperalgesia of the deep tissues, and secondly, the degree to which the affected part cools when exposed to ordinary climatic conditions The role of vascular disturbances would thus become clear A reduced peripheral circulation predisposes to cold pain by allowing the part to cool, while an increased circulation relieves cold pain by preventing cooling Thus deep hyperalgesia does not necessarily give rise to cold pain This is particularly obvious in inflammatory conditions in which local warmth prevents cooling

In patients suffering from cold pain sympathectomy by abolishing reflex vasoconstriction interferes with the normal cooling of the extremity and so relieves spontaneous pain Unfortunately the deep hyperalgesia persists so that the patient is only partly relieved of his symptoms As might be expected, sympathectomy is of no value in these patients unless it results in a substantial and permanent reduction of cooling

All this, however, is still conjecture and many more observations are required to establish the facts   In particular it would be desirable to know the following facts about patients suffering from cold pain —to what extent does cold analgesia fail to develop in the affected part ? Is there some abnormality of deep cooling when the affected extremity is exposed to ordinary climatic conditions, and what effect does sympathectomy have upon this deep cooling ? Does sympathectomy occasionally affect the degree of deep hyperalgesia as well as altering the temperature of the part ?

Although all these questions remain unanswered, we have reported our findings at this preliminary stage with the object of stimulating further investigation of patients suffering from cold pain along these lines

On the relation of cold pain to causalgia   In the classical account of causalgia by Weir Mitchell (12) the pain was described as burning in character, felt mostly in the skin and aggravated by heat and dryness, while cold and moisture gave some relief   In addition, spasms of pain were induced by emotional disturbance, movements, noise and practically any external stimulus   The pain followed gunshot wounds of the peripheral nerves and was associated with a red glossy condition of the skin over the affected part   Since that time it has been generally recognised that the pain of classical causalgia is frequently relieved by sympathectomy   More recently there has been a tendency to apply the term causalgia to any painful condition which is improved by sympathectomy   Thus Ulmer and Mayfield (17) collected 75 cases of peripheral nerve injury in which pain was relieved by sympathectomy   Of these 38 appear from their account to have been cases of classical causalgia, while in 30 cases the pain was brought on by cold and relieved by warmth and was associated with a cold blue extremity In spite of the obvious difference between these two groups of cases they consider them all to be examples of causalgia

We wish to draw attention to the difference between classical causalgia as described by Weir Mitchell and many subsequent writers (2, 5, 9, 11, 14), and the clinical syndrome of deep hyperalgesia with cold pain that we have described in this paper , and we wish to point out that cold pain is not confined to nerve injuries but may occur with simple fractures and any other condition which gives rise to deep hyperalgesia of the extremities without a concurrent increase of local circulation   We think this distinction is desirable because it seems likely that the pain of causalgia is produced by a different mechanism (2, 9) from that causing cold pain, though both conditions may be improved by sympathectomy

SUMMARY AND CONCLUSIONS

1   Among the many patients suffering from painful conditions of the extremities there are a number in whom spontaneous pain occurs whenever the affected part is cooled   This phenomenon has been called " cold pain " and a number of patients with this symptom have been investigated

2 Cold pain was found to be deep and diffuse in distribution, and to have the same quality as pain arising from the muscles and joints In every case the deep tissues at the source of pain were abnormally sensitive to mechanical stimuli, and it is suggested that deep hyperalgesia with cold pain should be considered as a definite clinical entity

3 The effect of cooling upon deep pain sensibility has been investigated experimentally When the normal deep tissues are cooled rapidly spontaneous deep pain is experienced for a short time while the temperature is actually falling , at the same time analgesia of the deep tissues develops so that in spite of continued cooling the spontaneous pain fades away With slow cooling analgesia develops without appreciable spontaneous pain

4 If deep hyperalgesia is produced by the injection of calcium chloride, even slow cooling of the affected part gives rise to severe and prolonged pain, and the analgesia which normally accompanies cooling develops imperfectly unless the tissues are cooled to very low temperatures

5 It is suggested that a disturbance of this kind may be responsible for clinical cold pain, and the relation of cold pain to causalgia and vascular disturbances is discussed

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EVIDENCE ON THE ORIGIN OF LEIOMYOMATA OF THE SKIN OBTAINED BY PHARMACOLOGICAL STUDIES

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LEIOMYOMATA of the skin is an unusual disease   Stout (1) reviewed the world publications in 1937, finding 132 cases of multiple lesions, and 85 instances of solitary tumors   He added 18 cases of his own   Prior to Stout's paper, but three reports had appeared in English (2, 3, 4)

Smooth muscle tissue is found in many areas of the skin, especially in the pilomotor muscles and in the sweat glands   However, it is also seen about the genitalia (muscularis sexualis), the anus and axillæ, as well as in the areolæ and nipples of the breasts (muscularis areolæ and mammillæ) Tumors of smooth muscle tissue could conceivably arise from any of these areas, but their origin has not been demonstrated in any of the recorded cases   Histological study has not contributed to this problem

It is the purpose of this paper to present a method whereby the origin of multiple skin leiomyomata may be determined   It is based on the responses of such tumors to mechanical and pharmacological stimuli

Case Report

E C , a 55 year old white housekeeper, was admitted to the Grady Hospital, May the 26th, 1947, for study of multiple skin nodules   She had been known to have diabetes mellitus since 1934   Shortly afterwards, multiple subcutaneous nodules appeared over the face, neck, arms and torso to the level of the costal margins   These nodules first appeared as painless papules, but gradually developed to the size of 1 cm , at which time they became painful   The pain was described as occurring in paroxysms, often rhythmically, with concommitant contraction of the lesions   There was often associated nausea and vomiting, and fecal and urinary urgency Her symptoms were aggravated by changes in room temperature, by mechanical irritation of the lesions, and by emotional stress   They were also more severe at times of increased glycosuria   New lesions appeared and developed throughout this thirteen year period   None of the lesions had ever become infected or exhibited evidence of hemorrhage or ulceration

Throughout her life, the patient had noted excessive perspiration She had always been asthenic in build, but had lost no weight during the period of the present illness Her diabetes had never been well controlled She maintained an almost constant glycosuria, and, in 1939, developed diabetic acidosis associated with a urinary tract infection At the time of admission, she was taking 35 units of protamine zinc insulin and 15 units of regular insulin daily

The patient's father, and a sister and niece were reported to have multiple skin nodules similar to those of the patient

Physical examination The temperature, pulse and respirations were normal The blood pressure was 150 mm mercury systolic, and 75 mm diastolic The patient did not appear acutely ill, but was apprehensive and easily excited Her skin was generally moist and warm Scattered over face, neck, arms and body down to the level of the costal margins were approximately 125 raised nodules, lying chiefly in the subcutaneous tissues, but including also the structure of the skin proper A single nodule was found on the right leg The nodules varied in size from 3 to 10 mm in the largest diameter They were round, elliptical or egg-shaped in contour, and appeared generally pink or violaceous Stimulation of the lesions by pressure or striking resulted in paroxysms of pain and contraction, associated with blanching and wrinkling of the overlying skin and puckering of the surrounding skin

The remainder of the physical examination was not pertinent

Laboratory data Repeated urine examinations revealed a trace to 3+ albuminuria, and negative to 4+ sugar reaction The white blood cell count was 6,600 per c mm , with a normal differential count The blood hemoglobin concentration was 14 g per 100 c c The blood sedimentation rate was 34 mm in one hour The fasting blood sugar level was 172 mg per 100 c c

Biopsy Several lesions were removed surgically from the anterior chest wall Histological examination showed them to be typical leiomyomata

Method

The local response of this patient's tumors was determined following both mechanical stimulation and subcutaneous administration of sympathomimetic and cholinergic drugs All tests were performed with and without preliminary sedation in order to minimize emotional factors

The criteria adopted as indicating a positive response to stimulation were contraction and shortening of the tumor mass, blanching and wrinkling of the overlying skin, and puckering of the surrounding skin

Mechanical stimulation was done by striking, pinching or pricking a tumor with a pin   The effect of temperature was determined by raising or lowering the room temperature, and by local application of heat or cold

Pilocarpine nitrate, acetyl choline and mecholyl hydrochloride were administered subcutaneously in doses sufficient to produce marked salivation and perspiration   These drugs were again given following the administration of atropine sulphate in doses large enough to produce dryness of the mouth, diminution of perspiration, and a decrease in intestinal peristalsis   The effects of atropine and adrenaline hydrochloride (0 3-1 0 mg) were determined independently of other drugs   The patient also received test doses of calcium gluconate (10 c c of a 10 per cent solution, intravenously) and curare (Intocostrin, 50-100 units, intramuscularly)

Tests were performed on this patient over a period of sixteen days When more than one test was performed in the course of a day, adequate time was allowed between tests for the effects of previously administered drugs to subside completely   During the period of observation, the patient continued to show a mild to moderate glycosuria   At no time was there evidence of diabetic acidosis or hypoglycemia

Results

Stimulation of the lesions by striking, pinching or pin-pricking produced sharp pain followed by a typical contraction   This contraction persisted over a period of one and a half minutes, and did not recur   Local cooling yielded the same results   Exposure to a room temperature of 41°F similarly caused pain and contraction of the lesions   Local application of heat and wrapping in blankets resulted in increased redness, but no pain or contraction The administration of atropine did not alter these responses

TABLE I

Response of skin leiomyomata to drug stimulation

Drug	Dose	Response of Lesions	Response of Lesions after Atropinization (0 6—1 2 mg atropine sulphate)
Pilocarpine nitrate	5 — 15 mg	+	0
Acetyl choline	25 — 50 mg	+	0
Mecholyl hydrochloride	5 — 15 mg	+	0
Adrenaline hydrochloride	0 3 — 1 0 mg	0	
Atropine sulphate	0 6 — 1 2 mg	0	

The results of drug administration are shown in Table I Pilocarpine, acetyl choline and mecholyl all produced marked painless contraction of the lesions It is noteworthy that these contractions were rhythmical, occurring one to two minutes apart The effects of these drugs were completely abolished by previous atropinization The injection of adrenaline or atropine alone resulted in no subjective or objective changes in the lesions Intravenous calcium gluconate and intramuscular curare (Intocostrin) were similarly without effect

The administration of phenobarbital (0 1 gm) or codeine sulphate (0 064 gm) in no way altered the results of any of the tests

Discussion

Of the various location of smooth muscle tissue in the skin and its associated structures, only two are sufficiently widespread to concern us here These are the pilomotor muscles and the smooth muscles of the sweat glands Both these groups of muscles receive their nerve supply from the autonomic nervous system The pilomotor muscles contract in response to sympathin and sympathomimetic drugs The sweat glands receive fibres from the sympathetic chains, but it is known that these fibres release not sympathin but acetyl choline, and the glands respond to artificial stimulation by cholinergic drugs The tumors of the patient under discussion responded to cholinergic drugs, the response being completely abolished by previous atropinization Adrenaline was without effect The distribution of the tumors is also of considerable interest Of the 126 lesions found, only one was outside the area supplied by the cervical sympathetic chain It has been shown by List and Peet (5) that the sweating produced by pilocarpine and mecholyl is largely confined to this area The location of this patient's lesions, and their sensitivity to cholinergic drug stimulation is considered conclusive evidence that they arose from smooth muscle tissue of sweat glands

Hagiwara and Sugizaki (6) reported that skin leiomyomata responded to the injection of adrenaline by painful contractions It is quite possible that these tumors arose from pilomotor muscles

Three members of the patient's family are reported to have multiple nodules of the skin None of these has noticed pain or contraction of the lesions, and a definite diagnosis is not available in any of them There is no reported instance of a familial tendency in this disease

Summary

1 A case of multiple leiomyomata of the skin is presented The functional response of these tumors to mechanical stimuli and sympathomimetic and cholinergic drug stimulation has been studied

2 The sensitivity of these tumors to cholinergic drugs and their distribution within the area supplied by the cervical sympathetic nerves is considered conclusive evidence of their origin from smooth muscle tissue of sweat glands

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CARDIAC OUTPUT AND PERIPHERAL BLOODFLOW IN ARTERIOVENOUS ANEURYSM *

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In their early work on arteriovenous aneurysm, Lewis and Drury (6) came to the conclusion that the cardiac output was probably not increased   This conclusion was based on the observation that the peripheral venous pressure did not change when the aneurysm was closed   Acute experiments in dogs showed that the cardiac output did not increase unless the shunt was exceptionally large   Holman (3) disagreed with this conclusion and demonstrated in experimental animals that the cardiac output and heart size increased in parallel   The development of the increased cardiac output, however, might be a matter of hours or longer, as heart size decreased immediately after the experimental shunt was opened

Kennedy and Burwell (4) studied cardiac output in one case of arteriovenous aneurysm using the acetylene method, and concluded that the output was increased and that it returned to normal following operation

Lewis and Drury made observations on forearm bloodflow using the venous occlusion plethysmograph without occluding the circulation to the hand , they observed that the bloodflow in the forearm increased when a femoral aneurysm was closed, and they ascribed this to the rise in mean blood pressure   On the other hand, Tournade and Goinard (8), observing increased oscillometer deflections on closure of the shunt, suggested that vasodilatation might also be present   Lewis and Drury also considered that, with the aneurysm open, the resting bloodflow in the forearm was less than in normal individuals   Lewis (5) later observed in long-standing cases that oscillometer deflections were increased and that bloodflow measured by the Stewart calorimeter was also increased in affected limbs distal to the shunt

Material and methods The observations were made on 12 cases, all males All had been wounded less than 2 years previously except Case 10 who was wounded 5 years before, and Case 12 whose aneurysm had been present for 29 years Cardiac output and right auricular pressure were studied by the technique of cardiac catheterisation, as previously described (7), and the patients were supine at the time of observation Since the weight of the subjects varied considerably, cardiac output has been expressed as litres per min per 100 c c oxygen consumed (normal average 2 2 litres) Bloodflow was measured in the forearm and in the leg, using the venous
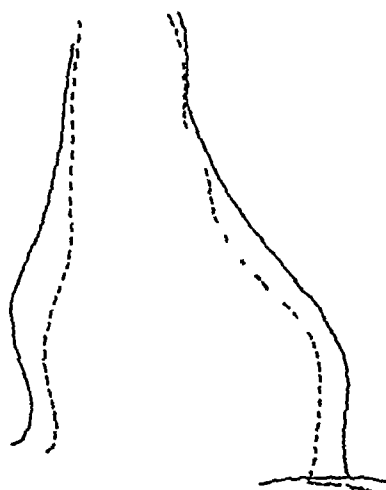
Fig 1 Effects of surgical closure of left popliteal arteriovenous fistula on heart size The dotted line shows the heart size 6 weeks after operation, when cardiac output had fallen from 3 7 to 2 7 litres per min per 100 c c oxygen consumed and the blood pressure had risen from 134/58 to 136/90 Pre operatively, the transverse diameter was 14 6 cm and cardiac area 155 sq cm, post operatively, the transverse diameter was 13 0 cm and the cardiac area 132 sq cm

occlusion plethysmograph (1) The arm was immersed in a waterbath at 34°C, while the leg was in air at room temperature (1) Circulation through the aneurysm was shut off by digital pressure proximal to the aneurysm In order to allow time for the circulation to reach a steady state, closure was maintained for one minute before the right auricular samples were withdrawn Bloodflow changes in the limbs were recorded within 30 sec of closure of the shunt

Results

Cardiac output

The results are shown in Table I Cardiac output in litres per minute per 100 c c oxygen consumed was increased above the normal average of 2 2 in nearly all cases, the highest figure being 4 5 in a case with a very large

TABLE I

Effects of closure of an arteriovenous aneurysm

Case No	Age	Site of aneurysm		Right auricular pressure cm saline above sternal angle	Cardiac output l per min per 100 cc O₂ consumed	Blood pressure, mm Hg	Heart rate	Oxygen consumption cc per min at room temp
1	20	L common carotid and L int jug vein	Shunt open	—1 0	4 5	120/58	88	357
			Shunt closed	—1 5	3 1	120/82	72	
			After atropine					
			Shunt open	—2 5	6 7	126/58	104	
			Shunt closed	—4 5	4 0	—	96	
2	32	L femoral artery and vein	Shunt open	+3 5	4 1	118/55	84	296
			Shunt closed	+2 0	2 7	115/70	54	
			After atropine					
			Shunt open	0	7 9	124/55	112	
			Shunt closed	—2 0	6 4	134/90	100	
3	20	R axillary artery and vein	Shunt open	0	3 9	124/70	84	366
			Shunt closed	—0 5	2 5	120/84	64	
4	34	L femoral artery and vein	Shunt open	—0 5	3 4	122/60	86	322
			Shunt closed	—2 0	2 3	128/80	60	
			After atropine					
			Shunt open	—4 0	3 2	122/66	114	
			Shunt closed	—6 5	2 8	138/98	100	
5	25	L axillary artery and vein	Shunt open	—5 0	3 4	125/60	84	330
			Shunt closed	—7 0	2 0	125/75	68	
6	36	L popliteal artery and vein	Shunt open	—3 5	3 7	134/58	70	367
			Shunt closed	—	2 4	136/86	60	
7	19	R femoral artery and vein	Shunt open	+1 5	3 3	120/62	84	250
			Shunt closed	+0 5	2 5	120/70	68	
8	25	L femoral artery and vein	Shunt open	—1 0	3 1	104/58	78	272
			Shunt closed	—2 0	2 4	118/78	65	
9	—	Innominate artery and L innom vein	Shunt open	—5 5	2 8	130/70	90	310
10	25	L subclavian artery and vein	Shunt open	0	2 4	98/55	55	265
			Shunt closed	—0 5	2 1		46	
12	51	R femoral artery and vein	Shunt open	+0 5	5 0	128/78	76	243
			Shunt closed	—0 5	3 45	145/80	64	

aneurysm (Case 1)   The venous filling pressure and heart rate were moderately increased

Cardiac output is probably raised by two physiological mechanisms, firstly, the increased filling pressure of the heart and, secondly, cardiac acceleration   Both these factors, however, are only moderately increased The effect of a summation of the changes in heart rate and venous filling pressure of the order found in the cases described has not yet been studied in young normal adults, so that it is impossible to say whether any further factor is involved

Teleradiograms made before and after operation showed a significant decrease in heart size when the shunt had been permanently closed   An example of conspicuous change in heart size is shown in Fig 1   The increase in heart size in arteriovenous shunts may be due to a raised stroke output and, in long-standing cases, to some hypertrophy, for which increased cardiac work may be responsible

Effects of acute closure of shunt   When the shunt was closed by digital occlusion of the artery proximally, the venous filling pressure fell slightly and the heart rate slowed considerably in all cases (Table I)   The diastolic arterial pressure also rose   Cardiac output fell but did not reach normal average values   Release of the arterial occlusion was followed by the return of these various measurements to their previous values

There is probably a close relationship between the size of the aneurysm and the cardiac output   In the case with the smallest shunt seen at operation, the cardiac output was only slightly increased (Case 7), whereas the patient with a free communication between the nearly severed carotid and the jugular vein had the highest output (16 0 litres per min)   Those subjects with the highest cardiac outputs showed the greatest decrease in output on closure of the shunt (Table I)   The question of whether the measured decrease represents the actual volume of blood flowing through the shunt is still doubtful   When the shunt was closed, the fall in right auricular pressure was small, and this did not appear to be due to accumulation of blood in the affected limb distal to the shunt, since limb volume decreased (Fig 2)   The reduction in cardiac output on closure is more closely correlated with the decrease in rate, which is considerable, than with the change in filling pressure (Fig 3)

With large arterial leaks, cardiac output values remained higher on digital closure of the shunt than those observed some time after operation Thus, there seems to be some slow circulatory adjustment following operation   It has been suggested that this is due to a fall in blood volume (2), but other factors, such as decline in ventricular hypertrophy, or changes in venous tone, may have to be taken into account

Effects of atropine   Atropine, given to three cases in an intravenous dose of 2 mg , accelerated the heart rate to a level of 104 to 114 beats per
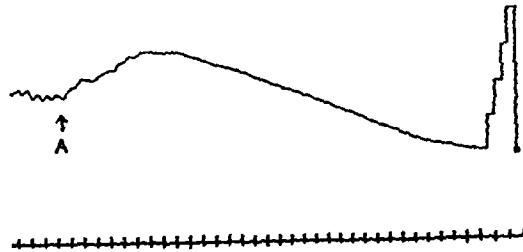
Fig 2 To show the changes in limb volume distal to a femoral arteriovenous aneurysm when the femoral artery was occluded digitally proximal to the shunt at A. There was a small increase in volume followed by a decrease Limb volume was 1400 c c Calibration in 5 c c steps Time in seconds
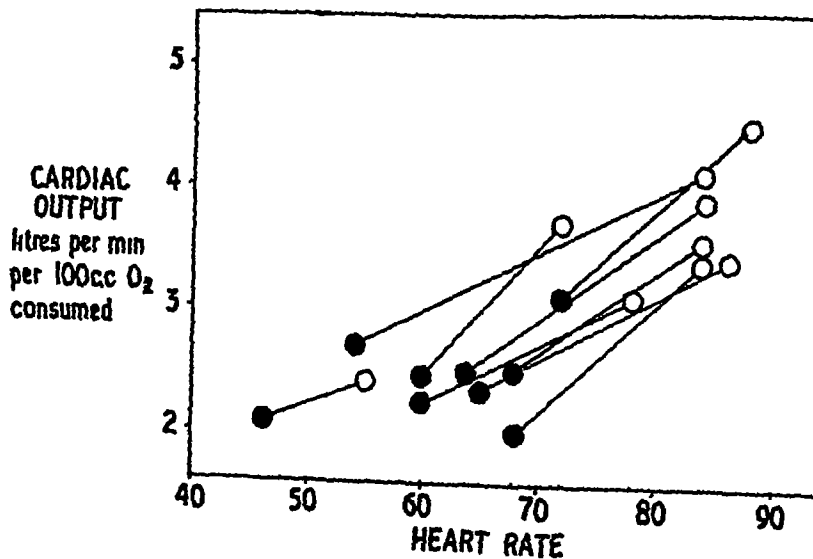
Fig 3 To show the relationship between changes in cardiac output and changes in heart rate on closure of arteriovenous aneurysms White discs—aneurysm open, black discs—aneurysm closed Case 12 omitted

minute The acceleration was accompanied by a considerable rise in cardiac output in two cases, although the right auricular pressure fell by 1 5 to 3 5 cm When a steady level of right auricular pressure was reached, closure of the shunt produced less showing than before atropine, the venous pressure fell by 2 to 2 5 cm and the cardiac output also decreased It is possible, however, that full atropinization was not attained in these three cases It should be noted that the very high cardiac output levels observed after atropine were more liable to errors of measurement owing to the small arteriovenous oxygen differences

Effects of reducing venous pressure by cuffs on the thighs In Case No 5 (Fig 4), right auricular pressure was lowered by congesting cuffs on the thighs to the same extent as after closure of the shunt This procedure caused a very small rise in heart rate The cardiac output fell by only 15 per cent of the resting value, or considerably less than the 40 per cent change after closure of the aneurysm This observation affords further evidence that the decrease in cardiac output on closure of the shunt depends more on heart rate than venous pressure changes
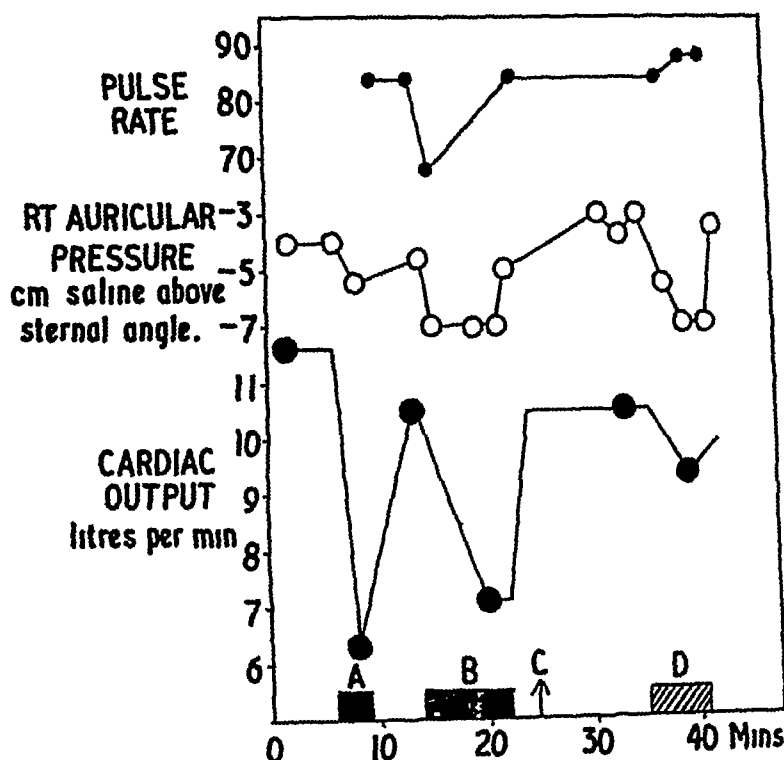
Fig 4 (Case 5) Axillary aneurysm
Effects of closing the shunt compared with the effects of lowering right auricular pressure by cuffs on the thighs At A and B the shunt was closed Cuffs placed on the thighs at C were inflated to 60 mm Hg at D There was a greater fall of cardiac output on closure of the shunt than on lowering right auricular pressure to a similar level by cuffs

Effects of surgical closure In 3 cases cardiac output was studied following recovery from operation (Table II) In 2 cases, the cardiac output had fallen to a considerably lower level than that produced by acute closure of the aneurysm The right auricular pressure had also fallen to normal values In Case 6 the cardiac output and right auricular pressure did not differ significantly following operation from the levels on acute closure of the shunt, but in this patient the popliteal arteriovenous communication was found to be small

TABLE II

Post-operative results

Case No		Right auricular press cm. saline above sternal angle	Cardiac output l per min per 100 c c oxygen consumed	Blood pressure	Heart rate	Oxygen consumption c c per min
1	Before operation	—1 0	4 5	120/58	88	357
	2 mths after operation	—4·0	2 3	125/80	68	363
2	Before operation	+3 5	4 1	118/55	84	296
	1 mth after operation	—3 5	2 0	120/75	58	277
6	Before operation	—3 5	3 7	134/58	70	367
	6 wks after operation	—4 0	2 7	135/90	62	352
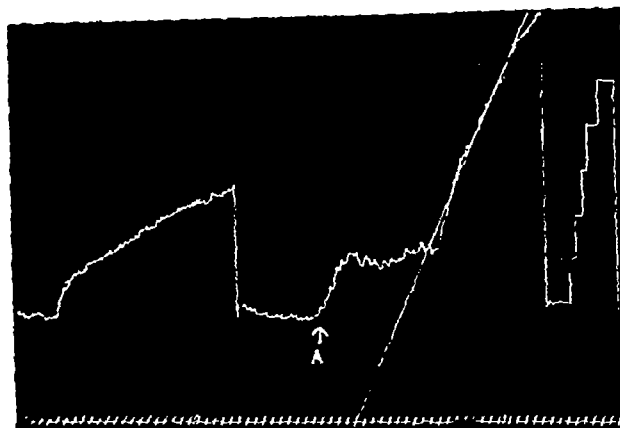
Fig 5 (Case 4) Left femoral aneurysm
On the left is the flow in the left forearm with the aneurysm open (2 4 c c per 100 c c per minute) On the right the aneurysm was closed at A This was followed by a volume increase and 12 sec after closure the recorded flow shows a conspicuous increase to 8 9 c c per 100 c c per min Arm volume was 580 c c Calibration in 2 c.c steps Time in seconds

2 Peripheral bloodflow

Bloodflow in limbs unaffected by the shunt Results are shown in Table III With the shunt open, the average forearm flow was 3 2 c c per 100 c c

forearm per minute At the bath temperatures employed this average flow is not significantly lower than normal (1) In one subject with a low forearm flow (Case 1), a similar finding was observed several weeks after operation, suggesting that the low flow was not related to the presence of

TABLE III

Forearm and leg bloodflows before and after acute closure of shunt

Case No	Site of aneurysm Time since wounding	Limb under observation		Bloodflow (c c per 100 c c per min)	Blood pressure mm Hg	Pulse rate per min
3	R axillary	R forearm	Shunt open Shunt closed	2 7 0	124/70 120/84	84 64
	less than 2 years	L forearm	Shunt open Shunt closed	4 6 6 6	"	"
		L leg	Shunt open Shunt closed	1 6 2 5	"	"
5	L axillary	L forearm	Shunt open Shunt closed	1 0 0 15	125/60 125/75	84 68
	less than 2 years	R forearm	Shunt open Shunt closed	1 7 2 6	"	84 68
10	L subclavian	L forearm	Shunt open	1 5	98/55	55
	5 years	R forearm	Shunt open	1 5	"	"
2	L femoral	R leg	Shunt open Shunt closed	1 5 2 6	118/55 115/70	84 54
	less than 2 years	R forearm	Shunt open Shunt closed	4 4 9 9	"	"
4	L femoral	R forearm	Shunt open Shunt closed	2 1 5 5	122/60 128/80	86 60
	less than 2 years	L forearm	Shunt open Shunt closed	3 3 9 0	"	"
8	L femoral less than 2 years	R forearm	Shunt open Shunt closed	5 2 7 1	104/58 118/78	78 65
7	R femoral less than 2 years	L forearm	Shunt open Shunt closed	2 6 3 4	120/62 120/70	84 68
1	L carotid less than 2 years	R forearm	Shunt open Shunt closed	1 6 2 4	120/58 120/82	89 72
11	R ulnar less than 2 years	L forearm	Shunt open Shunt closed	3 1 2 7	126/58 —	58 54
12	L femoral	L leg	Shunt open Shunt closed	8 0 5 3	128/78 145/80	76 64
	29 years	R leg	Shunt open Shunt closed	3 6 6 15	"	"

an aneurysm (Table V) In one other case in which flows were measured before and after operation, no change was recorded Thus in an unaffected limb, bloodflow through muscle is essentially normal In Lewis and Drury's observations where skin bloodflow was a large component, a subnormal flow was observed

On closure, the flow rose (Fig 5) to an average of 5 0 c c per 100 c c per min The increase in flow was observed in all cases except Case 11, in which there was a small ulnar arteriovenous communication The increase in flow occurred immediately the shunt was closed These results are in agreement with the findings of Lewis and Drury (6) Limb volume

TABLE IV

Effects of nerve block

Case No	Site of aneurysm		Forearm flow (c c per 100 c c per min)	
			Control	Blocked
2	L femoral	Shunt open	4 4	8 4
		Shunt closed	9 9	9 9
4	L femoral	Shunt open	R 2 1 L 3 3	L 4 1
		Shunt closed	5 5 9 0	6 5

TABLE V

Post-operative results

Case No	Time since operation	Site of aneurysm	Blood flow (c c per 100 c c per min)	
			normal limb	affected limb
2	1 month	L femoral	1 7	1 7
			2 4	2 5
			6 0	6 5 after indirect heating
1	2 months	L carotid	1 8	

also increased immediately on closure of the aneurysm and only returned to the original level when the shunt was reopened

Effect of nerve block in limbs unaffected by the shunt In two cases, a procaine block of the median, ulnar and radial nerves was carried out The bloodflow was measured in both forearms, one with the nerves blocked
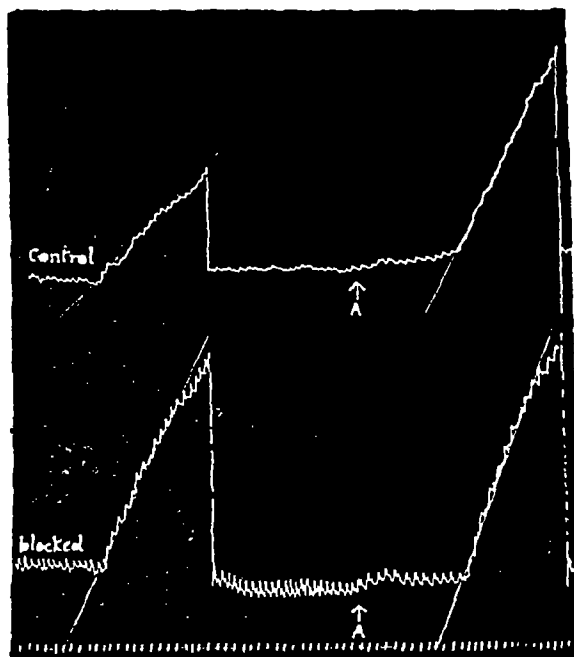
Fig 6 (Case 2) Left femoral aneurysm
The upper tracing shows first the flow in the control arm with the aneurysm open (4 9 c c per 100 c c per minute), and then the flow in the same arm when the aneurysm was closed at A (8 5 c c per 100 c c per minute) The lower tracing shows similar flows in the other forearm in which the radial, median, and ulnar nerves had been blocked by procaine After nerve block, closure of the shunt caused only a slight increase in forearm bloodflow (from 9 1 to 10 2 c c per 100 c c per minute) Time in seconds

and the other normal Acute closure of the shunt then caused an increase in flow in the arm with nerve block which was much less conspicuous than that seen in the normally innervated arm (Table IV, Fig 6) and the change in limb volume was much reduced

The bloodflow through a limb may be considered as depending on the blood pressure and the calibre of the vessels, and Lewis and Drury suggested that the increase in peripheral flow on closing the shunt was due to the rise of blood pressure The small increase in flow in a limb in which the vaso-motor nerves had been blocked might well have been caused by the rise of mean blood pressure The conspicuous increase in flow in a normal limb,

however, may have been due mainly to a reflex release of vaso-constrictor tone or active vasodilatation

Bloodflow in limbs affected by the shunt In 4 cases the bloodflow was measured in the affected limb distal to the shunt (Table III, Cases 3, 5, 10 and 12) In Cases 3 and 5, wounded less than 2 years previously, the flow was considerably less than in the normal contralateral limb, 1 0 c c compared with 1 7 c c , and 2 7 c c compared with 4 6 c c In Case 10, wounded 5 years previously, the flows on the two sides were similar When the shunt was closed, the flow decreased to very low or unrecordable levels in the first 2 cases (Fig 7) In Case 12, however, wounded 29 years previously, resting bloodflow in the affected limb distal to the aneurysm was considerably greater than in the normal leg, 8 0 compared with 3 6 c c per 100 c c of leg
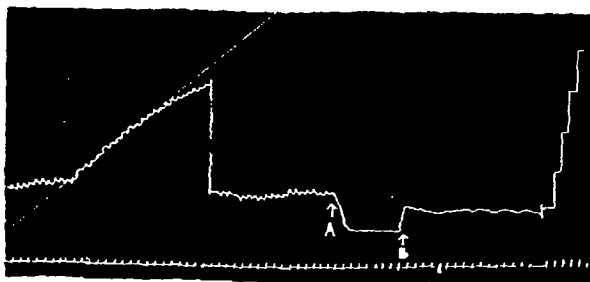
Fig 7 (Case 3) R axillary aneurysm
Forearm bloodflow was unrecordable when the collecting cuff was inflated at B after closure of the shunt at A Limb volume was 710 c c Calibration in 2 c c steps Time in seconds

per min In contrast also with the preceding cases, when the aneurysm was closed, leg bloodflow, though diminished, was still at the high level of 5 3 c c per 100 c c of leg per min

It has been thought that the bloodflow in the affected limbs peripheral to the shunt was increased, because of the raised skin temperature and, in some cases, the greater growth of the limb (2, 3 and 5) The data given above support Lewis' (5) view that the phenomenon of increased bloodflow beyond the shunt is only to be observed in long standing chronic cases

In the early cases, the negligible or absent bloodflow in the affected limb when the shunt was closed by digital compression of the artery just proximal to the aneurysm would seem to indicate that nearly all the blood supply to the distal portion of the limb flowed along the affected vessel past the aneurysm The reduced flow was not likely to be due to spasm of the collateral vessels, since bloodflow returned to the original level immediately the occluding pressure was released It seems unlikely that there was any conspicuous development of collateral vessels in these early cases

In contrast, the one long-standing case studied showed a greater bloodflow through the affected limb distal to the shunt with the aneurysm closed than was present in the contralateral normal resting limb, suggesting a considerable development of collateral vessels

Post-operative observations The results in the 2 cases studied are shown in Table V Bloodflows in the limbs unaffected by the shunt were unchanged from pre-operative levels Bloodflow in the limb in which the aneurysm had been removed by quadruple ligation and excision in Case 2 was similar to the contralateral limb, and responded in a similar manner to the release of vasomotor tone by indirect heating (Fig 8)
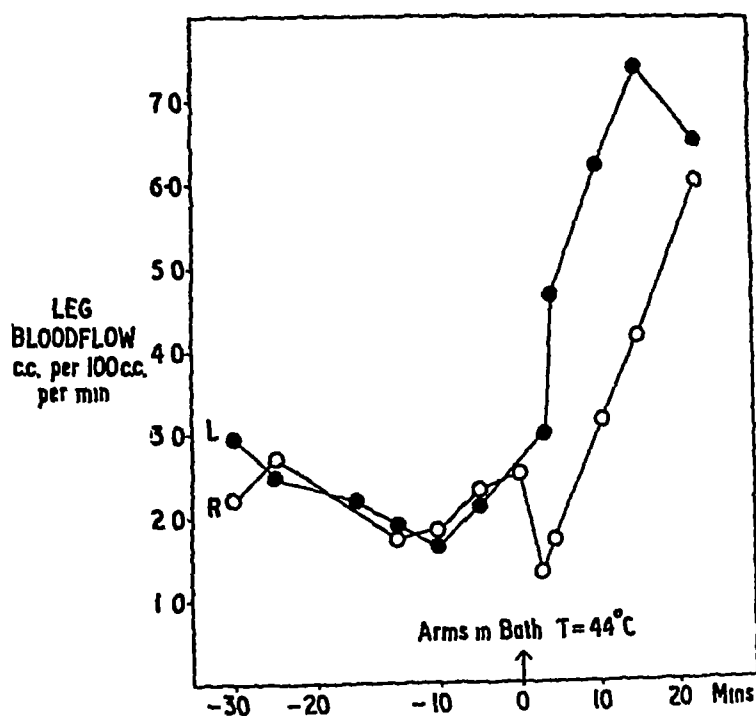
Fig 8 (Case 2) Left femoral aneurysm
    The effect of indirect heating on leg flows 30 days after quadruple ligature on the left side
    There is no difference on the two sides Black discs—left leg , white discs—right leg

SUMMARY

1 Cardiac output was increased in large arteriovenous aneurysms and the increase had some relationship to the size of the communication Right auricular pressure and heart rate were moderately increased

2 Closing the arteriovenous fistula by compressing the artery proximal to the shunt, produced slowing of the heart rate, increase in the diastolic

blood pressure, a considerable decrease in cardiac output and a small decrease in right auricular pressure   In large shunts, however, neither cardiac output nor right auricular pressure fell to normal levels on closure

3   2 mg atropine intravenously increased cardiac output to high levels (up to 24 litres per min), but changes in rate, cardiac output and auricular pressure were still produced by acute closure   Output changes on closure are more related to heart rate than to filling pressure changes

4   The bloodflow in limbs unaffected by the shunt was within normal limits and showed a conspicuous increase on closing the shunt   This increase was greatly reduced by procaine block of the mixed nerves to the limb, suggesting that the increased flow was mainly due to vasodilatation and only in part produced by the rise in mean blood pressure

5   Bloodflow in the affected limb distal to the arteriovenous fistula was reduced in lesions up to 2 years, normal in lesions of 5 years, and conspicuously increased in a lesion of 29 years duration   The flows recorded after compression of the artery proximal to the fistula suggested that in early lesions most of the blood entering the distal part of the limb traverses the aneurysm, while in long-standing lesions much arrives through other arterial channels

6   Resting bloodflow in a limb one month after quadruple ligature was the same as in the normal contralateral limb, and the vascular responses to raising body temperature were identical

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THE EFFECT OF 2-THIOURACIL ON THE CREATINURIA OF THYROTOXICOSIS, AND ITS USE IN THE DIAGNOSIS OF HYPERTHYROIDISM

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ADULT men do not normally excrete significant amounts of creatine in the urine, yet creatine is regularly excreted by children, and is occasionally present as a physiological constituent in the urine of women   In certain conditions, however, creatinuria occurs in both sexes at any age

Shaffer (15) was the first to report creatinuria in cases of Graves's disease, and this has been generally confirmed and accepted   Krause and Cramer (9) and many subsequent workers have shown that the administration of thyroid or thyroxine is followed by a brisk creatinuria   Palmer (11) and others were able to diminish and even suppress thyrotoxic creatine excretion by giving iodine   Thyroidectomy is soon followed by cessation of creatine excretion   It has been suggested that the effect of iodine is not *specific* and may be due to the improvement in the general health of the patient

2-Thiouracil is recognised as being able to inhibit the thyroid secretions This paper reports the effects on creatinuria of giving thiouracil to subjects with experimental and spontaneous thyrotoxicosis, and to subjects with creatinuria not due to hyperthyroidism

Methods and material

Urine was collected under rigid supervision, and creatine and creatinine were estimated daily from samples of the 24-hour collection (Folin (6)) A control period of 5 to 10 days was allowed before administering thiouracil The ordinary hospital diet was supplied   Experience has shown that a creatine free diet in hospital patients is unnecessary, as the normal diet does not significantly affect the results

The preparations used were 2-Thiouracil, and thyrotrophic extract which was prepared by Organon Laboratories (each ampoule is described as

containing 200 Heyl-Laqueur units per ml) The patients investigated
were —

(a) Normal subjects with no evidence of muscle or endocrine disease

(b) Patients believed to be thyrotoxic on clinical grounds, with a
     raised B M R and creatine excretion in the urine

(c) Patients with changes in circulatory dynamics similar to those in
     thyrotoxicosis, who were also excreting creatine

(d) Control patients with creatinuria due to conditions other than
     thyrotoxicosis

(e) Patients with evidence of thyroid overactivity, but not excreting
     creatine
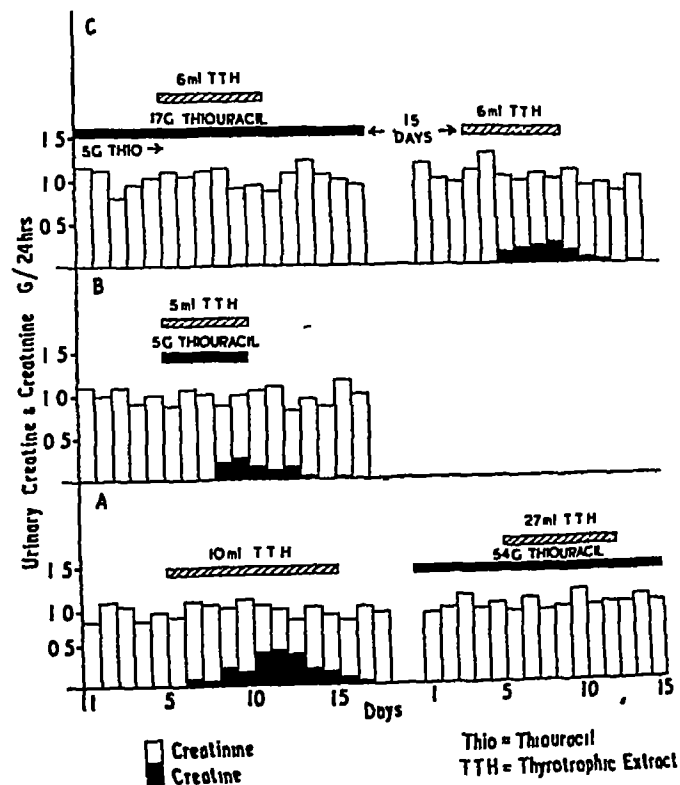
Fig 1   Effects of thyrotrophic extract and thiouracil on creatine excretion in normal subjects
   A   Thyrotrophic extract was injected, 1 ml a day for 10 days, and creatine was excreted
       2 0 g thiouracil was given for 18 days, and, while continuing the thiouracil, thyrotrophic
       extract was injected   There was no creatinuria
   B   1 ml thyrotrophic extract was injected for 5 days, and simultaneously, 1 g thiouracil
       was given per day for 5 days   A mild creatinuria was produced
   C   Thiouracil was given 1 g /day for 5 days   While continuing the thiouracil, 1 ml /day
       of thyrotrophic extract was injected for six days   There was no creatinuria   Thiouracil
       was stopped and, 15 days later, 6 ml thyrotrophic extract was injected in daily doses
       of 1 ml   A mild creatinuria occurred

Effect of — thiouracil on the creatinuria of thyrotoxicosis

Patient	Age	Sex	Average BMR at start % normal	Maximum creatine g per 24 hr	Minimum creatine g per 24 hr	Average creatine g per 24 hr	? thiouracil g per 24 hr	Total thiouracil to suppress creatine, g	No of days to suppress creatinuria
1	55	F	+52	0.23	0.14	0.18	1.0	7.0	7
2	42	F	+05	0.54	0.21	0.37	2.0	18	9
3	47	F	+38	0.39	0.18	0.31	0.5	7.5	15
4	23	F	+35	0.30	0.11	0.32	0.5	4.8	8
5	00	F	+01	0.13	0.14	0.20	1.0	0	0
6	43	F	+16	0.16	0.04	0.2	0.5	4.2	7
7	58	F	+42	0.28	0.08	0.13	0.5	5.4	0
8	22	M	+20	0.2	0.04	0.1	1.0	6.0	6
9	53	F	+32	0.31	0.08	0.23	1.0	6.0	0
10	03	M	+44	0.22	0.00	0.16	1.0	7.0	7
11	30	M	+0	0.2	0	0.00	1.0	4.0	4
12	74	F	+35	0.54	0.25	0.32	1.0	10	10
13	60	F	+33	0.43	0.27	0.31	1.0	8	8
14	45	F	+43	0.15	0.3	0.35	1.0	7	7
15	03	M	+4	0.18	0	0.00	0.8	30	5
16	38	M	+25	0.10	0	0.08	0.0	18	3
17	40	F	+18	0.20	0.05	0.12	0.0	12	4
18	44	F	+40	0.10	0	0.00	0.0	4.8	8
19	33	F	+48	0.32	0.05	0.18	1.0	7	7
20	24	F	+22	0.18	0.08	0.1	0.5	7	11

Results

The effect of 2-thiouracil on creatinuria in experimental thyrotoxicosis in normal subjects   Thyrotrophic extract was used to produce the experimental thyrotoxicosis   Fig 1 shows the results obtained by (a) thyrotrophic extract given alone, (b) thyrotrophic extract given after a
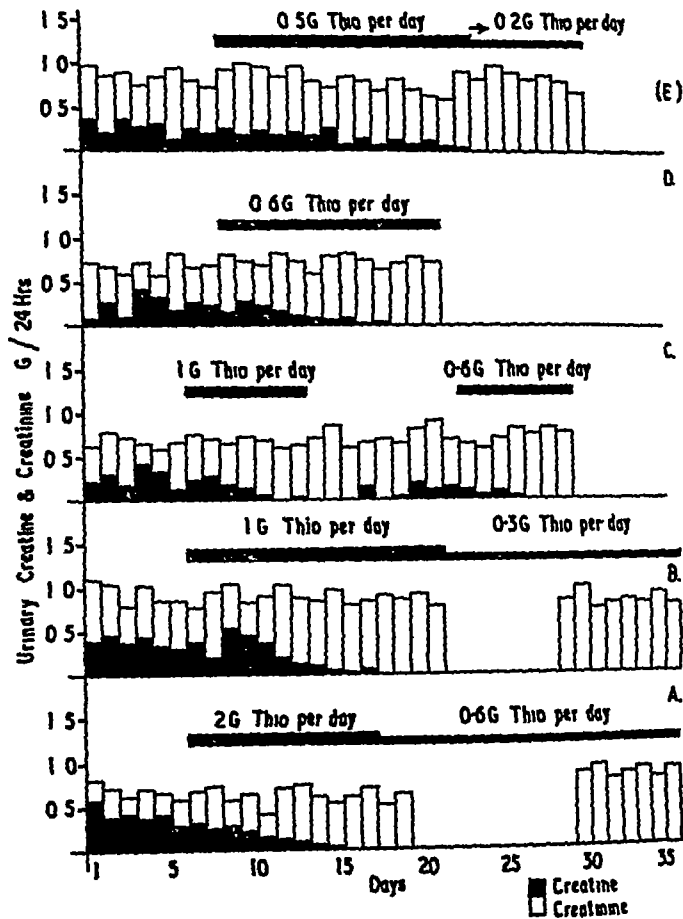
Fig 2   Effect of 2 thiouracil on creatinuria in thyrotoxicosis

    A   *Marked creatinuria*   2 0 g thiouracil per 24 hr   Creatine was suppressed in 10 days (18 g)   A maintenance dose of 0 6 g per 24 hr prevented recurrence of creatinuria

    B   *Marked creatinuria*   1 0 g thiouracil per 24 hr   Creatine was suppressed on the 10th day (10 g)   A maintenance dose of 0 3 g per 24 hr prevented recurrence of creatinuria

    C   *Moderate creatinuria*   1 0 g thiouracil per 24 hr   Creatine was suppressed on the 6th day   Thiouracil was stopped on the 8th day and seven days later creatine was again excreted   A dose of 0 6 g thiouracil per 24 hr stopped the renewed creatine excretion in 4 days

    D   *Moderate creatinuria*   0 6 g thiouracil per 24 hr   Creatine was suppressed on the 9th day

    E   *Moderate creatinuria*   0 5 g thiouracil per 24 hr   On the 15th day creatine was suppressed.

short course of thiouracil, and (c) thyrotrophic extract given simultaneously with thiouracil

Subject A   No creatine was excreted in the control period   Thyrotrophic extract was given for 10 days, 1 ml per day, and the expected creatinuria occurred (13) (17)   The subject was then given 2 g thiouracil per day for 18 days (36 g) and, while continuing the thiouracil, thyrotrophic extract was injected as shown   27 ml injected in 7 days failed to produce a creatinuria

Subject B   1 ml thyrotrophic extract was given simultaneously with 1 g thiouracil per day for 5 days   Creatine in small quantities was excreted in the urine

Subject C   1 g thiouracil per day was given for 5 days, and then 1 ml per day of thyrotrophic extract was injected for 6 days while the thiouracil was continued   No creatinuria occurred   The thiouracil was stopped, and 15 days later 6 ml thyrotrophic extract was injected at the rate of 1 ml per day   Creatine was excreted in the urine

Another subject who already had received 90 g thiouracil administered at the rate of 2 g per day, was injected with 34 ml thyrotrophic extract in 11 days while the thiouracil was continued   No creatine was excreted The extract used was from the same batch and was potent in normals

Effect of thiouracil on the creatinuria of thyrotoxicosis   Twenty cases of thyrotoxicosis were investigated, and the results are summarised in Table I In all these patients creatine was excreted every day and the quantities excreted are recorded in the table   Fig 2 shows the effects of various doses of thiouracil on the creatinuria in 5 selected cases

One patient was given 2 g thiouracil per day   On the 10th and following days no creatine was excreted   A maintenance dose of 0 6 g per 24 hours prevented a recurrence of the creatinuria

Ten patients received doses of 1 0 g thiouracil per day   The average total amount of thiouracil required to suppress creatinuria was 6 8 g   This occurred within an average of 7 days   Seven patients received doses of 0 6 g thiouracil per day   The average total amount needed to suppress creatine excretion was 4 3 g   This occurred within an average of 7 days Two patients received 0 5 g thiouracil per day   Creatine excretion was suppressed within 15 days in one, and 14 days in the other   The amount of thiouracil given was 7 5 g and 7 g respectively

It was found that a maintenance dose of 0 3 g thiouracil per 24-hr was sufficient in all degrees of creatinuria to prevent a recurrence of creatine excretion   A dose of 0 2 g was sufficient in the milder cases

Fig 3 presents the results of plotting the average daily excretion of creatine against the total dose of thiouracil needed to suppress the creatinuria   There is a distinct relationship between the total suppressing dosage of thiouracil and the creatinuria   In three cases thiouracil was stopped several days after creatine excretion had been suppressed   In one

TABLE II

Effect of thiouracil on creatinuria not due to hyperthyroidism Creatinuria is unaffected

Patient	Age	Sex	Clinical diagnosis	Goitre	B P	Average daily creatine g per 24 hr	Thou racil g per 24 hr	Total thou racil, g	Average daily creatine after thiouracil	Effect of thiou racil on creatinuria
1	60	M	Ischaemic heart disease	0	145/85	0 06	1 0	11	0 06	Nil
2	58	F	Congestive failure	0	168/98	0 06	1 0	10	0 05	Nil
3	71	F	Ischaemic heart disease	0	210/110	0 10	1 0	14	0 10	Nil
4	65	M	Congestive failure	0	195/136	0 07	1 0	12	0 05	Nil
5	72	F	Hypertensive heart disease Failure	0	210/108	0 05	1 0	12	0 08	Nil
6	58	M	Hypertensive heart failure	0	190/105	0 07	1 0	10	0 08	Nil
7	60	F	A fibrillation Hypertensive heart disease Failure	+	168/80	0 07	1 0	10	0 06	Nil
8	45	F	Hypertensive heart failure	+	158/98	0 06	0 8	5 4	0 05	Nil
9	70	F	Hypertensive heart failure	0	180/100	0 06	1 0	9	0 05	Nil
10	64	M	Hypertensive heart failure	+	195/110	0 05	1 0	14	0 06	Nil
11	47	M	Hypertensive heart failure	0	168/110	0 07	1 0	10	0 06	Nil
12	55	F	Hypertensive heart failure	+	210/105	0 05	1 0	10	0 05	Nil
13	62	M	Hypertensive heart failure	0	190/100	0 10	1 0	10	0 11	Nil
14	60	M	Hypertensive heart failure	0	180/105	0 08	1 0	12	0 06	Nil
15	55	M	Carcinomatosis	0	130/75	0 19	1 0	12	0 16	Nil
16	58	M	Carcinomatosis	0	158/98	0 10	1 0	10	0 18	Nil
17	48	M	Carcinomatosis	0	180/100	0 04	1 0	5	0 05	Nil
18	56	M	Carcinomatosis	0	165/95	0 13	1 0	10	0 12	Nil
19	60	F	Reticulosis	+	160/95	0 32	1 0	10	0 30	Nil
20	44	F	Acromegaly	+	155/85	0 33	2 0	54	0 32	Nil
21	42	M	Acromegaly	+	158/80	0 32	2 0	38	0 41	Nil

case creatine was once more excreted 7 days later, and was then rapidly suppressed in 4 days by resumption of a daily dose of 0 6 g thiouracil

In the other 2 cases creatine excretion recurred within 4 and 8 days respectively after stopping thiouracil In the first case, thiouracil was stopped two days after creatinuria had been suppressed, and in the second case 5 days after suppression of creatine excretion
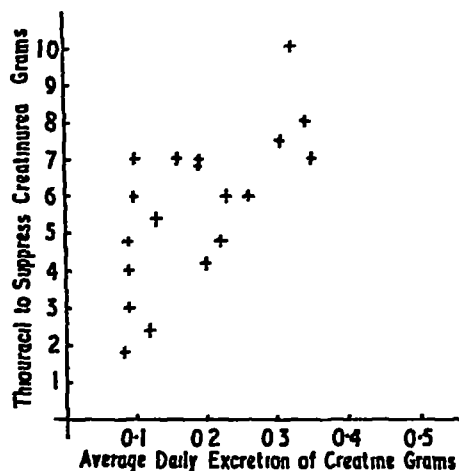
Fig 3
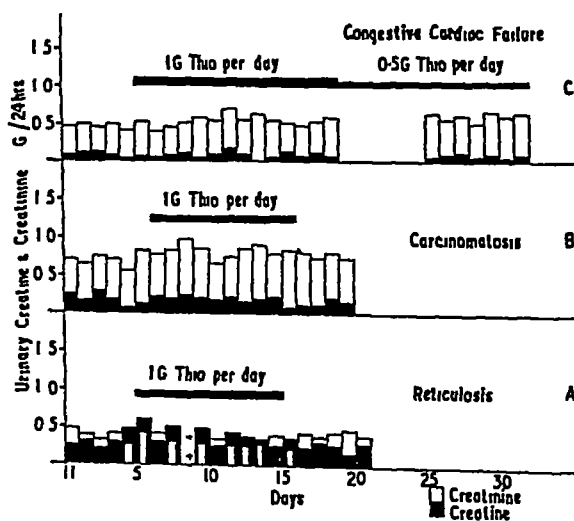
Fig 4 The effect of thiouracil in 3 patients with no thyrotoxicosis excreting creatine every day The creatinuria is not abolished by thiouracil

TABLE III

Seventeen patients referred as cases of hyperthyroidism   None excreted creatine in the urine

Patient	Age	Sex	Av BMR %	B P	Heart rate	Circulatory state	Goitre	Eye signs	Creatin uria	Remarks
1	32	M	+21	140/95	84	Normal	++	Nil	Nil	Histology Non toxic goitre / Partial sup V caval block / Later in failure and passed creatine
2	58	F	+28	158/90	86	Aortic incomp / Aortic aneurysm	+	Nil	Nil	Not in failure
3	47	F	+31	135/85	72	Aortic stenosis / A fib	+	Nil	Nil	
4	42	F	+12	128/79	88	Normal	0	Exophth lid lag Nil	Nil	Thyroidectomy 8 years ago / Nervous Tremors+ / Nervous
5	28	F	+17	134/80	76	Normal	+	Nil	Nil	Nervous Jumpy
6	26	F	+ 5	124/80	80	Normal	+	Nil	Nil	Headaches
7	65	M	— 7	174/88	78	Normal	+	Exophth hd lag Nil	Nil	Weakness and tired
8	47	M	+50	150/85	94	Normal	+	Nil	Nil	Hist Non toxic goitre / Nervous
9	35	F	+ 7	140/85	08	Normal	+	Nil	Nil	Nervous
10	40	F	+ 7	92/50	86	Hyp ht failure	+	Nil	Nil	Retrosternal goitre
11	67	F	+20	205/110	88	Normal	+	Exophth	Nil	Tremors
12	35	F	+ 4	116/66	74	Normal	+	Lid lag	Nil	Hyperventilation tetany / Nervous
13	48	F	— 4	145/90	76	Normal	+	Nil	Nil	Dyspnœa, bronchitis and emphysema Tremors / Nervous
14	60	F	+11	100/70	90	Normal	0	Nil	Nil	Nervous Tremors
15	42	F	+ 1	150/84	84	Normal	++	Nil	Nil	Nervous Tremors
16	22	M	+12	140/80	80	Normal	+	Nil	Nil	Thyroidectomy 1939 / Losing weight
17	71	M	— 8	136/08	08	Normal	0	Exophth	Nil	

Effect of thiouracil in patients without thyrotoxicosis excreting creatine

Table II shows the results obtained in 21 cases in whom creatinuria was present Fourteen patients were in congestive heart failure, and of these five had enlargement of the thyroid gland In some of these patients, the circulatory changes were similar to those in thyrotoxicosis, but the daily creatinuria was less than 0 15 g and was not present every day Thiouracil in daily doses of 1 g for 10 to 15 days had no effect on the creatine excretion Five patients had generalised carcinomatosis and wasting Creatine excretion was marked in 4 of these patients Thiouracil in doses of 1 g
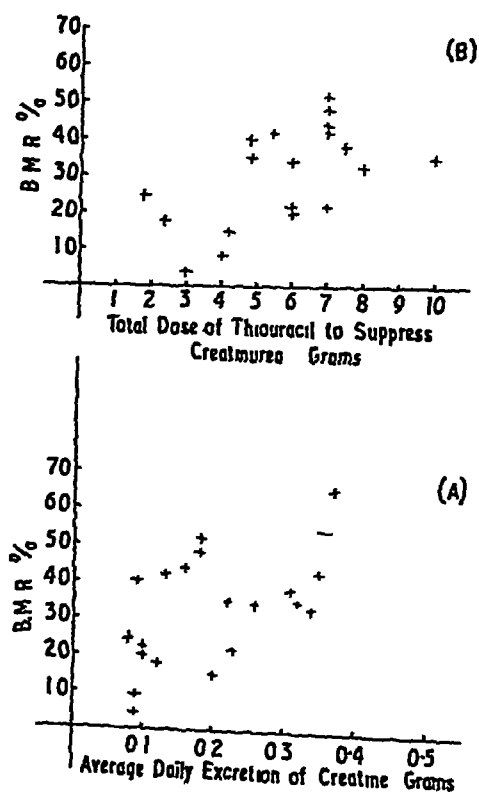
Fig 5

for 10 to 14 days had no effect on the creatinuria Two patients with acromegaly had enlargement of the thyroid and marked creatinuria One was given 2 g thiouracil per day for 27 days (54 g), and the other 2 g per day for 14 days (28 g) Creatinuria was unaffected.

Fig 4 presents graphically the results obtained in 3 selected patients excreting creatine every day, and the effect of thiouracil on the creatinuria

*Investigation of cases thought to be thyrotoxic on clinical grounds but not
excreting creatine*   Seventeen cases who were thought clinically to be mild
or inactive cases of hyperthyroidism were investigated   A raised B M R,
enlargement of the thyroid, or a circulatory disturbance were the presenting
features   Thirteen of these patients had thyroid enlargement, but none
excreted any creatine in the urine for the 14 days or more under investigation
Table III summarises the results obtained in this group   It is very difficult
to be certain how many of these patients actually had thyrotoxicosis   In
spite of clinical evidence of thyrotoxicosis and a raised B M R, two of these
cases, 1 and 8, showed histologically non-toxic goitres from material obtained
at operation

Table IV records a summary of the results in all the 71 patients
investigated

<div align="center">TABLE IV</div>

Summary of results in 71 patients investigated showing the effect of 2 thiouracil in creatinuria

Clinical diagnosis	No of patients	Thyroid enlargement	Creatinuria	Effect of thiouracil on creatinuria
Active thyrotoxicosis	21	18	21	Suppressed in 21
? Thyrotoxicosis	17	14	0	—
Congestive heart failure	14	5	14	No effect
Hypertensive heart disease (not in failure)	10	2	0	—
Malignant disease (wasting)	7	1	5	No effect
Acromegaly	2	2	2	No effect

<div align="center">DISCUSSION</div>

From the results obtained in normal subjects who were given thiouracil
and thyrotrophic extract, it is clear that thiouracil can prevent thyrotrophic
extract from producing a creatinuria   Giving 5 g thiouracil at the rate of
1 g per 24 hours before thyrotrophic injection, prevented the excretion of
creatine   The effect of thiouracil wears off quite quickly, for 15 days after
stopping it (the total given, 17 g), a creatinuria can be provoked by injecting
thyrotrophic extract   Once a subject has received 20 g of thiouracil or

more, even massive doses of thyrotrophic extract fail to produce a response, and the subject is then, as regards the thyroid response, in a similar condition to that seen in myxœdema where doses of 70 ml thyrotrophic extract or more fail to provoke a creatinuria

It is interesting to note that this refractoriness of the thyroid to thyrotrophic extract does not coincide with histological changes Doniach and Sharpey-Schafer (5) were not able to define clear cut histological changes in the thyroid, until 70 g or more thiouracil had been administered Their criteria of change were necessarily rigid, and it is to be expected that the physiological disturbance in thyroid secretion will precede the histological alterations in the thyroid gland

The results in thyrotoxicosis suggest that the more creatine excreted, the more severe is the degree of toxicity, and the more thiouracil will be required to suppress the excretion of creatine in the urine Fig 5A shows the results of plotting the average daily creatine excretion against the basal metabolic rates in the 21 patients Fig 5B shows the results of plotting the total dose of thiouracil needed to suppress the creatine excretion against the B M R There is a distinct relationship between the height of the B M R , the average daily creatine excretion, and the total dose of thiouracil needed to suppress the creatinuria, though in a small series of cases it is dangerous to draw conclusions

The best initial dose to suppress creatinuria proved to be between 0 6 g and 1 0 g per 24 hr Doses of more than 1 g are unnecessary Doses of 0 5 g and less are probably too small As 1 g of thiouracil per day suppressed creatinuria within 10 days, it is suggested that in the severe thyrotoxic patient this is the optimum dose In the less severe cases, 0 6 g per 24 hr for 10 days will be sufficient It is not necessary to estimate the creatinuria in thyrotoxicosis to decide on the dose of thiouracil to be given Clinical assessment of the case should be sufficient In doubtful cases, the presence of a persistent creatine excretion should be an indication for administering thiouracil The maintenance dose necessary to prevent the recurrence of creatine excretion, once it has been controlled, depends on the severity of the disease, and varies from 0 4 g per 24 hr in the severe cases, to 0 2 g in the moderate cases It is of interest that clinically, and by biochemical analysis, the beneficial effects of thiouracil are apparent before the B M R is depressed, and long before histological changes

The failure to prevent creatinuria in the two cases of acromegaly was surprising Not all acromegalics excrete creatine (3 and 13) but in those that do it has been postulated (13, 14 and 16), on experimental grounds, that the creatinuria is due to overstimulation of the thyroid by thyrotrophic hormone Both the cases investigated had enlargement of the thyroid and elevated B M Rs The failure to respond to large doses of thiouracil is unexplained Barfred (1) reported that methyl-thiouracil did not lower

the B M R in one case of acromegaly investigated   Rose and McConnell (12)
also failed to reduce the B M R in an acromegalic by methyl-thiouracil
given in doses of 0 2 to 0 6 g daily for 15 months

The diagnostic value of the creatinuria in thyiotoxicosis has been studied
by several workers   Kepler and Boothby (8) reported that in 145 cases of
hyperthyroidism, 61 % excreted creatine, but there was no strict parallelism
between the B M R and the creatinuria   Wang (20) has reviewed the
subject, and concludes that in thyrotoxicosis, creatinuria is not of diagnostic
help in assessing the grade of activity of the disease process   From the
study here made of 38 cases regarded clinically as thyrotoxicosis, it seems
that in thyrotoxicosis of a certain degree of activity, it is the rule for creatine
to be excreted every day, usually in quantities greater than 0 15 g per
24 hours   In patients suffering from thyrotoxicosis of a lesser degree,
creatine may not be excreted at all, though as the patients have been
investigated for only 3 weeks at a time, it is still possible that creatinuria
may be present at other times   The absence of creatinuria in a case suspected
of thyrotoxicosis cannot be taken as proof of normal thyroid function,
though it stiongly suggests that any thyrotoxicosis present is not severe

When the full clinical picture is present, thyrotoxicosis is easy to
recognise   The diagnosis may however be difficult, notably in those cases
with thyrotoxicosis without, or with doubtful, eye signs, and in those with
exophthalmos and its concomitants, but without the signs of hyper-
thyroidism   The B M R has been generally used both to diagnose and to
gauge the severity of the thyrotoxicosis   But an increase in the B M R
has been reported in a variety of conditions, and when this is small, it is
often difficult to be sure of its meaning   Mountain and others (10) analysed
837 cases of essential hypertension and reported that the B M R is greater
than +15% in 7% of the milder forms, increasing to 27% in the malignant
phases   They concluded that the raised B M R was not due to thyroid
disturbance   Weiss (21) compared the circulatory dynamics in essential
hypertension and thyrotoxicosis   He noted that the pulse rate, the velocity
and the amount of bloodflow are not increased in proportion to the
metabolism in essential hypertension with a raised B M R in contrast to the
state of affairs in hyperthyroidism   Crile (2) estimated the blood iodine
in 11 cases of hypertension with raised metabolism (and no hyperthyroidism)
and found normal values in 5, a slight elevation in 3, and abnormally high
values in 3   Curtis (4) found a raised blood iodine not infrequently in
essential hypertension, even without a raised B M R   Treusch and others
(19) compared the creatinuria of thyrotoxicosis and hypertensive heart
disease, and concluded from the different amounts excreted that the
mechanism of creatine production differed in the two conditions

It will probably only occasionally happen that the diagnosis remains
in doubt in a case of suspected thyrotoxicosis severe enough to exhibit
creatinuria, but when this is so the suppression of the creatine excretion

by thiouracil will be strong evidence in favour of its thyrotoxic origin   It has been shown that thiouracil can prevent the creatinuria provoked by thyrotrophic extract in normal subjects, and can abolish the creatinuria of thyrotoxicosis   It does not however affect the creatinuria of congestive heart failure and the other diseases here examined in which thyrotoxicosis is absent   These results suggest that the effect of thiouracil in suppressing creatinuria is only exerted where the creatinuria is due to thyroid overaction, and therefore this test may be useful in the diagnosis of doubtful cases of thyrotoxicosis in whom creatine is excreted
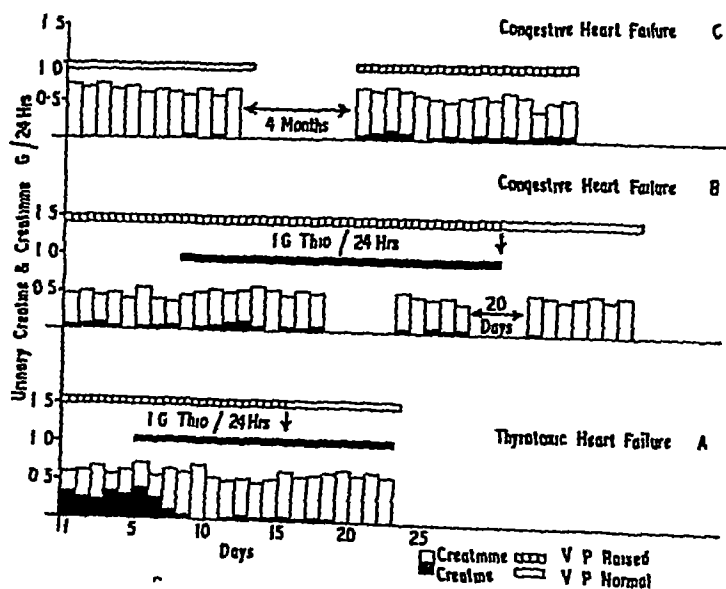
Fig 6   Creatinuria in thyrotoxicosis and heart failure

    A   *Thyrotoxic heart failure*   Creatinuria was not completely suppressed after thiouracil administration   as the patient was still in heart failure   When failure disappeared, and the venous pressure (V P) had returned to normal creatine was no longer excreted

    B   *Hypertensive heart failure*   The creatinuria was unaffected by thiouracil.   When heart failure had disappeared, and the thiouracil had already been discontinued, creatinuria ceased

    C   *Heart failure*   No creatine excretion when not in failure   Later the patient was investigated when she was in failure, and creatinuria was present

When there is a combination of etiological factors, as in thyrotoxic congestive heart failure, after giving thiouracil, the level of creatine excretion will drop markedly, but will not necessarily be suppressed   The residual creatinuria is due to the congestive heart failure   When the patient recovers from failure, creatine may no longer be excreted   Fig 6A presents the results of a typical example of this "two-stage" suppression of creatinuria   Fig 6B shows the results in a patient with congestive heart failure where

the creatinuria was unaffected by thiouracil Eventually creatine was no longer excreted when the patient had recovered from failure, and the thiouracil had already been discontinued Fig 6c presents the results in a case examined before and after congestive heart failure When first examined no creatine was detected in the urine for 22 successive days Four months later she was investigated in heart failure, and creatine was present in the urine on 13 out of 16 days The average daily amount excreted was 0 06 g

Why some patients with apparently clinically toxic goitres do not excrete creatine is not clear It is possible that the creatinuria, the raised B M R and the eye signs, such as exophthalmos, are not produced by the same factor Other workers have thought that the raised B M R and the creatinuria of congestive heart failure are not produced by the same mechanism as in hyperthyroidism Exophthalmos has been produced by thyrotrophic injection in thyroidectomised animals (7), but not in man Sharpey-Schafer and Rundle (personal communication) have injected as much as 100 ml thyrotrophic extract in a case of myxœdema with no subsequent changes in the eyes, while Schrire and Sharpey-Schafer (unpublished) have injected 56 ml thyrotrophic extract in experimental myxœdema, produced by thiouracil, without any observed effect on the eyes The extract used in each case was similar and 4 ml in normal controls produced active experimental hyperthyroidism

Creatine is derived from the muscles and can be mobilised by factors other than the thyroid gland secretion It is thus possible that the discrepancies in the results obtained in hyperthyroidism may be due to the fact that the raised B M R , the creatinuria and the eye signs of thyrotoxicosis are produced by separate hormones or mechanisms

Thiouracil may, with justification, be given to doubtful cases with a view to noting if there is any clinical improvement When this has been done, and improvement noted, the clinical amelioration has been presumed to be due to the inhibition of excessive thyroid activity Sharpey-Schafer (18) has, however, shown that thiouracil is a valuable drug in the treatment of congestive heart failure, and quite often in patients with no element of thyroid disease, improvement has been considerable Any clinical improvement after therapy with thiouracil cannot be diagnostic of thyroid disease in the group with congestive heart failure, whereas the suppression or not of creatinuria is objective and probably specific

SUMMARY

1 In normal subjects thiouracil prevented the excretion of creatine normally provoked by injection of thyrotrophic extract

2 Thiouracil suppressed creatinuria in thyrotoxicosis in 4 to 15 days, depending on the degree of creatinuria and the dose of thiouracil used

3   The suggested initial dose is 1 g per 24 hr for 10 days   The suggested maintenance dose is 0 3 g per 24 hr

4   Thiouracil did not suppress the creatinuria in other conditions investigated, including cases of congestive heart failure

5   In conditions where creatinuria is present and the diagnosis doubtful, it is suggested that thiouracil can be used as an aid to diagnosis

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TRANSFUSION OF SALINE-WASHED RED CELLS IN NOCTURNAL HÆMOGLOBINURIA

(MARCHIAFAVA-MICHELI DISEASE)

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NOCTURNAL hæmoglobinuria is an uncommon type of chronic hæmolytic anæmia, its course being generally measured in years Episodes of hæmoglobinuria, characteristically, but not always, nocturnal, alternate with periods of freedom lasting weeks or months during which time the hæmolytic process is less active, but still abnormal

The basic cause of the increased hæmolysis is an ill-understood abnormality of the patient's own red cells , they are sensitive to complement-like hæmolytic factors normally present in all human sera In vitro, the patient's washed cells can be shown to hæmolyse readily in his own serum and in that of normal individuals, if the pH is adjusted to the optimum for the activity of the serum factors (pH 7 2 to 7 4) No abnormal hæmolytic substance is present in the patient's serum or plasma as neither in vitro nor in vivo are normal cells hæmolysed more rapidly than normal

Some patients become seriously anæmic None of the known hæmopoietic substances is effective, splenectomy is uncertain in its effects and has been considered dangerous (5), while blood transfusion, an obvious remedy, has provoked hæmolytic reactions

The object of the present paper is to describe the beneficial effects of a series of transfusions with saline-washed red cells on a patient, previously reported (1), and to refer more briefly to a second patient similarly treated Further evidence will be presented in support of the hypothesis that it is the transfused plasma rather than the red cells that is responsible for the hæmolytic reaction which may follow transfusions Clinical details of the patients are given in an appendix

Methods

The amount of hæmoglobin in the urine was roughly estimated by naked eye inspection and scored as +, a definite reddish tinge, to + + +, deep red to black   Heparinized venous blood (0 1 mg heparin per ml) was used for the hæmatological studies   Red cells were counted in a 1 to 200 dilution, 20 c mm blood to 4 ml diluting fluid, at least 1000 cells being counted in each preparation   Reticulocytes were counted in films made from the deposit obtained by lightly centrifuging a suspension of cells in 0 15% cresyl blue in saline   Hæmoglobin (100% = 15 6 g), was estimated by the cyan-hæmatin method (7) and packed cell volume by using Wintrobe's hæmatocrit tubes centrifuged for 30 mins at 3000 r p m   Plasma bilirubin was estimated as described by King (6)

In vitro hæmolysis was studied by incubating at 37°C a 5% suspension of washed patient's cells in fresh serum acidified with 10% by volume of N/5 HCl   Samples withdrawn at intervals were immediately chilled and centrifuged   Hæmolysis was measured photoelectrically after conversion of the free hæmoglobin to alkaline hæmatin

The number of donor cells per c mm after transfusion was estimated, in the case of the patient L , by differential agglutination (2)   This patient was Group A Rh positive   The donors' blood (Group O Rh positive) was collected into acid-sodium citrate-glucose solution (100 ml to 440 ml blood) and stored at a temperature not exceeding 4°C for 5 to 12 days before use The blood was washed with sterile 0 85% saline in bottles of 150 ml capacity, the supernatant being removed by suction three times before the cells were finally suspended in saline up to a final volume of about 540 ml   Every effort was made to keep the blood samples sterile during these manipulations The transfusions were started as soon as washing had been completed, the blood being given in about 40 to 60 minutes

The effects of transfusing saline-washed red cells

Case 1, *Miss L*   This patient received the washed cells derived from 4,400 ml of blood during a period of 23 days   No subjective symptoms or clinical signs suggestive of an increased rate of hæmolysis developed except on a single occasion when two bottles of washed cells were given consecutively on the same day , in this case a moderate increase in jaundice was obvious on the following morning   The blood data are illustrated in Fig 1   The hæmoglobin content was raised from 50% to 102% (15 9 g), the red cell count from 1,700,000 per c mm to 4,700,000 per c mm and the packed cell volume (hæmatocrit) from 24 5% to 47%   The donor (un-agglutinable) red cell count rose to 3,600,000 per c mm   The patient's cell count (total red cell count less donor red cell count) fell gradually from 1,650,000 per c mm to a minimum of about 1,000,000 per c mm in 21 days   The reticulocyte count expressed as a percentage of the patient's cells fell from 37% to a minimum of 1 3%, within 4 weeks after starting the transfusions,

in absolute numbers from 610,000 per c mm to 20,000 per c mm The plasma bilirubin level had by then fallen from about 2 mgm per 100 ml to less than 0 5 mg per 100 ml The urine hæmoglobin initially present in large amounts throughout the day, and increased at night, showed signs
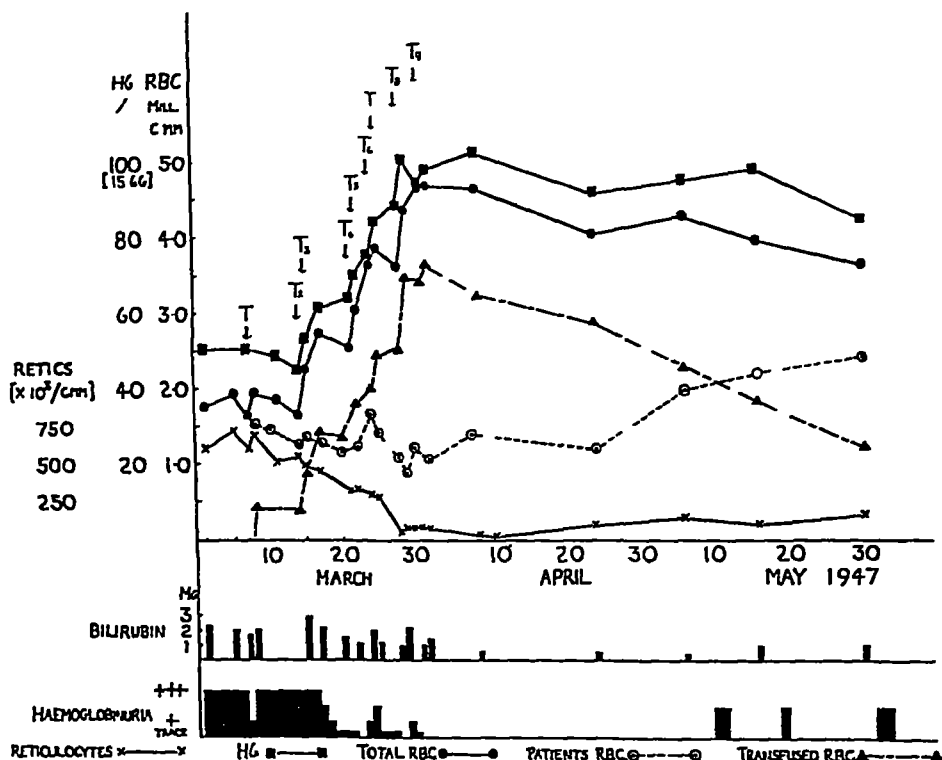
Fig 1 *Case Mrs L*, 1st admission and subsequent 2 months Blood and urine changes following multiple transfusions with saline washed red cells The day to day presence of hæmoglobin in the urine is recorded roughly quantitatively as black rectangles No black rectangle denotes freedom from hæmoglobinuria Hæmoglobin was raised from 50 to 102%, red cells from 1,800 000 per c.mm to 4,700 000 per c.mm The patient's cell count fell to 1 000 000 per c mm and reticulocytes from 610,000 to 20,000 per c.mm The transfused red cells survived well Note the disappearance of hæmoglobinuria *pari passu* with the fall in reticulocytes and the return of the plasma bilirubin to normal

of disappearing after the 3rd transfusion and was completely absent after the 8th transfusion

Sternal puncture was performed before the transfusions were started and 2 days after the last transfusion was given Stained smears and sections of the fragments aspirated showed reduced but still abnormally great erythropoietic activity in the second sample compared with the very active picture revealed by the first sample The patient left hospital on the 2nd

April feeling well, without jaundice or hæmoglobinuria and looking a normal healthy woman

The transfused cells were eliminated in a linear fashion   The un-agglutinable count had fallen to 50% of its maximum level in 45 days, as the blood samples varied in age from 5 to 12 days before being transfused and were subjected to considerable manipulation during the washing process this may be considered a normal survival

The hæmoglobin percentage fell gradually from a maximum of 102% on the 8th May to 82% on the 30th May   The total red cell count fell from 4,650,000 per c mm to 3,520,000 per c mm during this time whilst the patient's cell count rose from 1,050,000 per c mm to 2,340,000 per c mm The plasma bilirubin had by then risen slightly to 0 8 mg per 100 ml and there had been two short episodes of hæmoglobinuria

The patient was readmitted into hospital on the 1st June for further transfusions   Moderate nocturnal hæmoglobinuria had been present for 2 days   The washed cells of three further bottles of blood were given without subjective reaction and she was discharged from hospital on the 4th June The red cell count was then 4,150,000 per c mm and hæmoglobin 100%, her urine contained a small amount of hæmoglobin   Two days later (6th June) a brisk episode of continuous hæmoglobinuria developed which lasted until the 11th June   A blood count on the 13th June showed that the hæmoglobin had fallen to 81%, and that the patient's cell count had dropped from 2,000,000 per c mm to 1,770,000 per c mm   The un-agglutinable count had been raised to 2,150,000 per c mm as the result of the 2nd series of transfusions, the cells being eliminated slowly as before

She was readmitted on the 12th December, 1947, for further transfusions, having been free from hæmoglobinuria for the previous three weeks   Her hæmoglobin was then 68%   Details of the fluctuating course of the disease between June and December are given in the appendix

It seemed desirable to ascertain how important it was to wash blood free from plasma before transfusing, and to demonstrate, if possible, by in vitro tests the reason for this   On the 12th December, 20 ml of 8 day old plasma were given intravenously without provoking hæmoglobinuria, and on the day following approximately 65 ml plasma and 200 ml cells, taken from a bottle of 11 day old blood (T 1, Fig 2)   This transfusion had little or no effect on the total of patient's red cells and was not followed by hæmoglobinuria

On the 15th December, she received a transfusion of 13 day old blood, 420 ml cells and 80 ml plasma (T 2)   Soon after completion she complained of nausea and dizziness, and passed some almost black urine   An intense hæmoglobinuria persisted for 12 hours, but had disappeared within 24 hours A brownish tinge of jaundice developed, fading gradually after 2 to 3 days The transfused cells survived well and the hæmolytic episode seemed to be entirely due to destruction of the patient's own cells,   *the patient's cell*

count fell from 3,600,000 per c mm to 2,400,000 per c mm (Fig 2) She was none the worse for this hæmolytic attack, and subsequently was given three transfusions of saline-washed cells without reactions of any kind She was discharged from hospital with a hæmoglobin of 100%

One month later her hæmoglobin was still 95%, slight nocturnal hæmoglobinuria had, however, just started to return
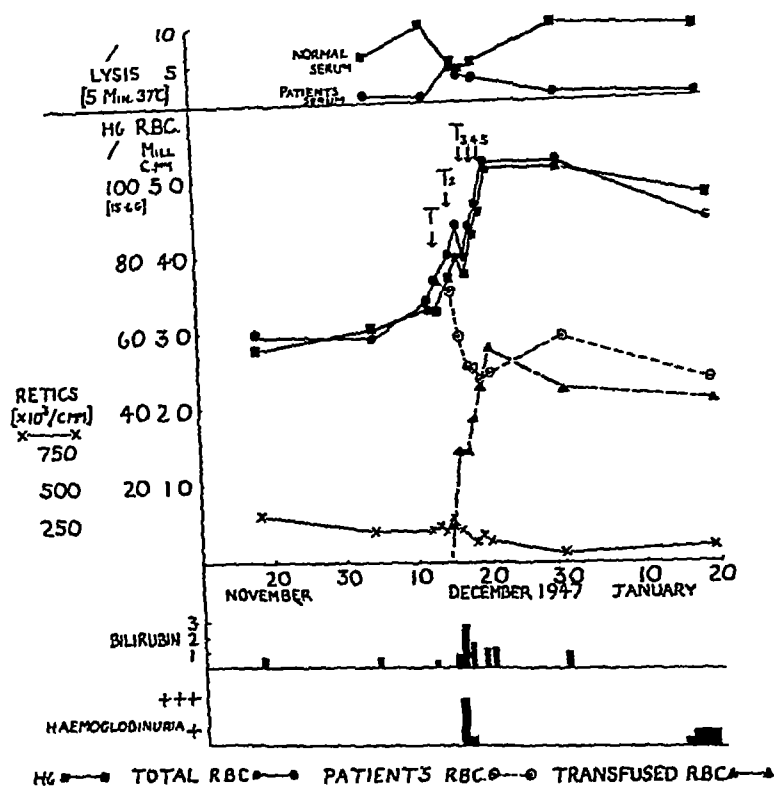
Fig 2   Case Miss L, 3rd admission and subsequent 1 month   Blood and urine changes following blood transfusions   The day to day presence of hæmoglobin in the urine is recorded roughly quantitatively as black rectangles   No record denotes freedom from hæmoglobin uria   The 3rd 4th and 5th transfusions (T 3 4, 5) were of washed red cells   The 1st and 2nd transfusion (T 1 2) were of unwashed packed red cells, the 2nd transfusion pre cipitated a hæmolytic episode with intense hæmoglobinuria during which time the patient s cell count fell from 3,600 000 per c mm to 2,400,000 per c mm   The total hæmoglobin was ultimately raised to 100%   The transfused cells once more survived well

Case 2, Mr A   This patient was given between the 14th August and the 3rd September, 1947, saline-washed cells derived from 4,400 ml of Group O Rh + blood   The blood changes are illustrated in Fig 3   On the 4th September his hæmoglobin was 106% and the *total red cells* 5,000,000 per c mm   His reticulocytes had fallen from 720,000 per c mm to 150,000

per c mm and the plasma bilirubin was then 1 1 mg per 100 ml   His
urine became free from haemoglobin on the 18th August and remained so
until his discharge from hospital on the 9th September   There was a
striking clinical improvement   He appeared to be a normal healthy man
except for slight icterus of the conjunctivae
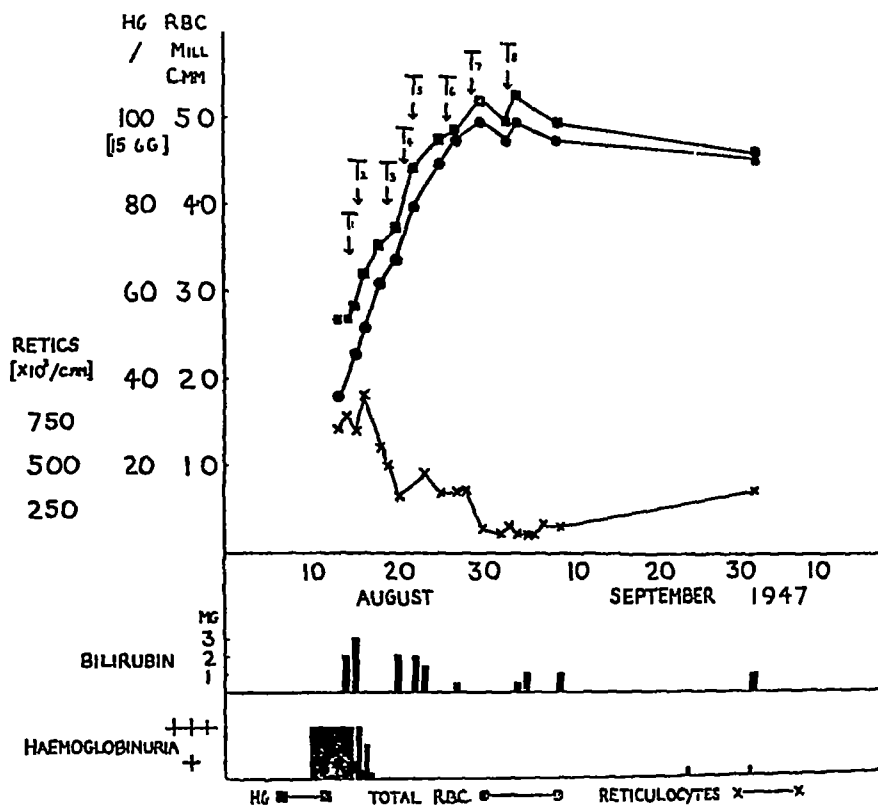
Fig 3   *Case Mr A*, 1st admission and subsequent 1 month   Blood and urine changes
following multiple transfusions with washed red cells   The day to day presence of
haemoglobin in the urine is recorded roughly quantitatively as black rectangles   No record
denotes freedom from haemoglobinuria   Total haemoglobin was raised to 100% and red cells
to 5,000,000 per c mm   The reticulocytes fell from 720,000 to 150,000 per c mm
Haemoglobinuria disappeared after the 2nd transfusion, and plasma bilirubin fell to about
1 0 mg per 100 ml

He was next seen on the 2nd October, 1947, four weeks after the final
transfusion,   he had been well since discharge from hospital   He had
passed urine containing haemoglobin on two occasions only   His red cell
count was 4,680,000 per c mm   and haemoglobin 93%   There were
370,000 reticulocytes per c mm   (7 8%)

He was readmitted into hospital on the 5th January, 1948   He
complained of weakness and jaundice and intermittent but slight haemoglo-
binuria   He was seriously anaemic,   red cells 1,550,000 per c mm with
haemoglobin 34%, and 40% reticulocytes (620,000 per c mm)   There was

1 mg bilirubin per 100 ml   His urine was free from hæmoglobin   He was transfused successfully and without reactions with the washed cells from 8 bottles of 2 to 7 day old Group O blood, divided into four transfusions at 2 day intervals   On discharge from hospital the red cell count was 5,400,000 per c mm , hæmoglobin 105%, reticulocytes 290,000 per c mm and bilirubin 0 9 mg per 100 ml   One month later (16th February, 1948) his red cell count was 4,700,000 per c mm and hæmoglobin 95%   He felt well and looked fit
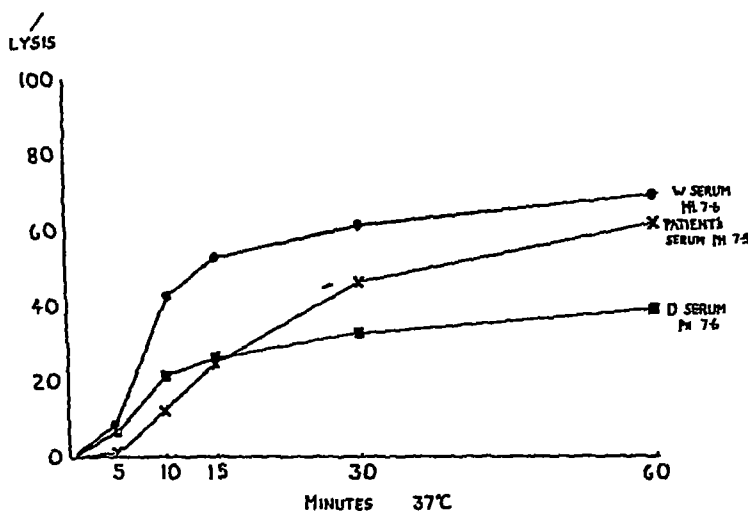
Fig 4   Time—hæmolysis curves of patient s cells in autogenous and two normal sera   Shows differences in hæmolytic power   W serum is most active   the patient's serum the slowest acting   Only a proportion of the patient s cells are hæmolysed

In vitro observations

The rate of hæmolysis of Miss L's cells in autogenous and normal acidified sera at optimum pH has been studied from time to time   It has been found that the ability of sera to hæmolyse patient's cells in vitro varies both in rapidity of action and in extent (Fig 4)   Before the hæmolytic episode which followed the red cell and plasma transfusion (T 2) given during her third admission, 1% or less of a suspension of patient's cells incubated in her own serum at 37°C for 5 min were hæmolysed, compared with 7 to 11% hæmolysed in 2 normal sera studied at the same time   During and soon after the hæmolytic episode 6 to 7% of cells were hæmolysed in the patient's own serum which was then as active as the normal control (Fig 2)

This experiment shows that of the three sera studied the patient's own serum was less rapidly hæmolytic than the two normal controls, and that during the hæmolytic episode its activity was increased   It was not possible to measure in vitro the hæmolytic power of the plasma transfused,

for the presence of citrate inhibits hæmolysis   It seems certain, however, that it was the plasma that caused the hæmolytic episode, and reasonable to suppose that this particular sample did contain hæmolytic factors capable of augmenting the activity of the patient's plasma—experiments showed that the activity of the latter was, in fact, temporarily increased

DISCUSSION

In 1943, Dacie and Firth described the effects of transfusions of serum and of packed but unwashed red cells on Miss L , one of the subjects of the present report, and briefly considered past writings on this aspect of the subject up to that time   Sometimes, but not invariably, there have been serious reactions   Sometimes, temporary remissions have been noted   In September, 1942, Miss L was given 5 successive transfusions of 400 ml of 6 months old stored serum without an appreciable effect on the blood count The regular rhythm of hæmoglobinuria at night with freedom during the day was, however, disturbed   Transient marked hæmoglobinuria immediately followed each serum transfusion   A subsequent transfusion with a concentrated suspension of 2-day old red cells in plasma (about 400 ml cells and 100 ml citrate-glucose plasma) precipitated a dramatic hæmolytic episode lasting 48 hours , more than half the patient's own red cells were destroyed with intense and continuous hæmoglobinæmia and hæmoglobinuria The patient recovered from this, and then had a remission from hæmoglobinuria lasting for 6 weeks, whilst the red cell count rose about 1 million per c mm   The transfused red cells survived well (data from Dr P L Mollison) and were eliminated slowly in a linear fashion , 22% of the donor cells present on the day after the transfusion were still circulating 98 days later, a normal survival

Compared with the alarming result of transfusing concentrated red cells, the effect of the 6 months old serum in comparatively large volumes was trivial , there were only minor episodes of hæmoglobinuria with no detectable change in the blood count

The difference was attributed to the comparative freshness of the plasma transfused with the concentrated red cells   In vitro, the 6 months old serum failed completely to hæmolyse a suspension of patient's cells, and its in vivo effect of provoking hæmoglobinuria was unexpected   That so much hæmolysis could be provoked by the transfusion of not more than 100 ml of 2-day old plasma seemed, however, very remarkable *

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* An increased sensitivity of patient's cells to hæmolysis with human isohæmolysin has been described (4)   Dacie and Firth (1) found that serum, although apparently free from isoantibodies, provoked hæmoglobinuria when given to Miss L , and therefore concluded that the isoantibody content of the serum transfused could not be the cause of the hæmolysis   Recently, the increased sensitivity of this patient's red cells has been reinvestigated and found to be unexpectedly great and it is now felt that the hæmolytic episodes which have followed transfusion of packed but unwashed group O red cells may have been due in part to an anti A isohæmolysin present in the plasma transfused

From the above observations it was thought that although transfusion with whole blood might be a dangerous procedure, it was the donor's plasma rather than the cells that was the cause of the hæmolysis of the patient's cells It seemed, moreover, probable that cells washed free from plasma might be transfused with safety, and because of the normal survival of the donors' cells that such a transfusion might be of considerable benefit to the patient The present report substantiates these conclusions

The improvement produced in both the above patients as the result of the present series of red cell transfusions was clinically striking , in both patients the hæmoglobinuria present at their first admissions dramatically disappeared *pari passu* with the rise in hæmoglobin   In the case of Miss L hæmoglobinuria had been present almost continuously in large amounts for 4 months , in the case of Mr A for 3 weeks   The more detailed studies carried out on Miss L indicate a significant diminution in the rate of hæmolysis and further support the evidence provided by the return of the plasma bilirubin to a normal level and the disappearance of hæmoglobinuria The number of reticulocytes fell from about 650,000 per c mm to 18,000 per c mm indicating a greatly reduced delivery of red cells from the bone marrow   The comparatively small fall in the patient's cell count from 1,650,000 per c mm to 1,000,000 per c mm taken in conjunction with this greatly diminished output from the marrow can only mean a reduced rate of hæmolysis

A debatable point is the relation of the transfusions to the remissions observed in both patients   Remissions occur spontaneously, and the failure of the relatively small transfusion at the beginning of June to abort an attack which seemed to be just beginning is a point against the invariable immediate efficacity of transfusion

There are two obvious possible mechanisms by which the rate of hæmolysis might be modified   Firstly, an alteration in the cells themselves There are differences in sensitivity between the cells of any given population in vitro, at least, some cells lyse quickly , other are resistant and do not lyse, even if the serum is changed several times   This is not connected with the age of the cells, for reticulocytes and adult cells are equally sensitive (unpublished observations)   Sensitivity varies also from case to case, and an increase or diminution in cell sensitivity may well play a part in determining the fluctuating course of the disease   Secondly, variation in plasma activity , this is almost certainly important and would well explain the sudden increases in hæmolysis associated with infections so frequently observed in these patients   This has already been referred to as the most probable explanation for the hæmolytic episodes which may follow transfusions containing plasma

However, no evidence is available which indicates that either of these mechanisms is the cause of the remissions now described following massive transfusions , the remissions following hæmolytic episodes (1) are at

least partly due to the sudden elimination from the circulation of a large proportion of the patient's most sensitive cells Nor is there evidence to support the hypothesis that the relief of anoxia following transfusion results in a diminution in haemolysis Moreover, the possibility that the mere admixture of normal with pathological cells will inhibit lysis of the latter is negatived by in vitro tests

The following explanation is believed to be, at least, partly responsible for the remissions now described The raising of the haemoglobin to the normal level with relief of anoxia results in a diminution in the rate of delivery of new cells from the bone marrow The fall in reticulocyte count is good evidence of this It follows from this that fewer abnormal cells will be available for haemolysis, and less blood pigment to be disposed of Haemoglobinuria will thus disappear and jaundice will be diminished, exactly as has been observed in the above two patients Relapse will, however, occur as the haemoglobin level slowly falls, due to the elimination of the transfused red cells An increasing output of abnormal cells from the marrow will result from this, and the patient's cell count will rise, a state of affairs suitable for a haemolytic episode, should the haemolytic activity of the patient's plasma be augmented for any reason In the case of patient L it was surprising to see that although the delivery of new cells from the marrow as revealed by the reticulocyte counts, had been drastically reduced, the patient's cell count only slowly fell from 1,650,000 to about 1,000,000 per c mm This observation is consistent with the in vitro finding of a proportion of cells distinctly resistant to haemolysis

SUMMARY

1   The raising of the haemoglobin level to the normal range by series of transfusions of washed red cells resulted in two patients in clinical and haematological temporary remissions The diminution in haemolysis is considered to be largely due to a reduced production of patient's cells, following relief of anoxia

2   The transfusion of saline-washed cells in these patients does not precipitate haemolytic reactions The transfusion of plasma may do so, probably by augmenting the haemolytic activity of the patient's own plasma

3   Normal cells survive well after transfusion Anaemia may thus be corrected by transfusion at intervals, and the patients in this way kept in a fair state of health

APPENDIX

Clinical observations

Case 1   *Miss L* The early history of this patient has been described previously (1) Her illness dates from November, 1940, when she was aged 36 Haemoglobinuria at night was the first sign of the disorder , later weakness and pallor with jaundice became conspicuous Nocturnal haemoglobinuria was diagnosed in 1942 All the signs of a chronic haemolytic anaemia

were present and the results of in vitro tests were characteristic , a proportion of her cells were hæmolysed by normal or autogenous serum if the pH was suitably adjusted (3)

Between December, 1942 and October, 1946, she gives a history of chronic illness and intermittent attacks of hæmoglobinuria generally lasting not longer than 10 days Some weakness and jaundice was persistently present to a varying degree She has repeatedly observed that mild infections of throat or chest and sometimes menstruation have seemed to precipitate episodes of hæmoglobinuria often severe enough to make her take to her bed, and from which recovery has generally been slow

When seen in October 1946, the hæmolytic process was more active than in 1942 , from October 1946 until her admission into Hammersmith Hospital on the 27th February, 1947, hæmoglobinuria had been almost continuously present, being always more marked at night time Her red cell count varied from 1,600,000 per c.mm to 1,900,000 per c.mm and hæmoglobin from 44 to 51% Reticulocytes ranged from 30 to 55% , mean cell volume averaged 140 cu μ The plasma contained oxyhæmoglobin and methæmalbumin and increased amounts of bilirubin In vitro tests were characteristic Her urine contained debris in which granules containing iron could be demonstrated by the Prussian Blue reaction Her spleen was just palpable but otherwise there were no significantly abnormal physical signs, other than anæmia and a slightly brownish type of jaundice

Her clinical course and the main blood changes between March and June, 1947, have been described in the text and illustrated in Fig 1

A clinical and hæmatological remission occurred in mid-June The hæmoglobin rose to 103% by the 11th July and the percentage of patient's cells in the form of reticulocytes fell to 2 3% Plasma bilirubin was normal and there was no hæmoglobinuria or visible jaundice She remained well until the 27th July when a brisk attack of almost continuous hæmoglobinuria developed which lasted until the 7th August The urine then remained clear until the 25th September Between this date and 8th December when she was again admitted into hospital, there was intermittent hæmoglobinuria mainly confined to night time Her hæmoglobin varied between 56 and 76% and there was slight jaundice

The data obtained during her 3rd admission are described in the text and illustrated by Fig 2

Case 2 Mr A This patient was a man aged 44 referred by Dr Beryl Baraby to this hospital for transfusion Hæmoglobinuria appeared first in September, 1946, but he had been unwell for 6 months previously During a second episode in January 1947, nocturnal hæmoglobinuria was diagnosed on the basis of in vitro tests On admission into Hammersmith Hospital (13th August, 1947) he was pale and jaundiced His spleen was just palpable but no other important signs were elicited His urine contained hæmoglobin at all times, and the urinary deposit many granules giving a positive Prussion Blue reaction A red cell count on admission revealed 1 800 000 cells per c.mm with 40% reticulocytes The hæmoglobin was 54% Mean cell volume was 145 cu μ bilirubin was 2 mg per 100 ml He was Group O Rh + Spectroscopic examination of the plasma when hæmolysis was active showed the presence of oxyhæmoglobin and methæmalbumin

The good response to transfusions with washed red cells during his first admission in August, 1947, has been described in the text By November, 1947, however, he was once more complaining of weakness and pallor He was readmitted into hospital in January, 1948 for further transfusions (*see text*)

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POSTURAL PROTEINURIA

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INTRODUCTION

PROTEINURIA is such a frequent and important sign of renal disease that the recognition of types which are harmless is of very great practical importance   Over a hundred years ago Becquerell (8) described a man who had albuminuria but was otherwise healthy   Since then a vast literature has grown up about the subject of the " Benign Proteinurias " but, despite this, there is no unanimity regarding many aspects   On the one hand there are some who deny the existence of benign forms of proteinuria (53, 54, 64, 61) while others consider that they are extremely common (24, 32, 44) Among those who accept that benign forms do exist, there is considerable difference of opinion regarding their incidence

It was soon recognised that most of the proteinurias occurring in otherwise healthy persons were intermittent   By the beginning of the twentieth century, certain postures, exercise and nervous factors were known to play a part in the production of the proteinuria but, even today, the importance of the various factors remains unsettled (50, 18, 51, 67)

Controversy regarding the pathogenesis of the proteinuria has existed since these cases were first recognised and even today there are several hypotheses to explain the various types   Many of the earlier ideas were based on an incomplete knowledge of the clinical picture and on concepts of pathology and physiology that are now outmoded   Hooker (29) reviewed the early work very completely and further references to it are to be found in articles by Fox (21), Nicholson (46) and Fishberg (18)   The hypotheses

that have survived to recent times can be grouped under four main headings based on —

1. The suggestion that there is an abnormality of the blood

2. The presumption that there are congenital or acquired lesions of the kidney and particularly of the glomeruli

3. Presumed vasomotor instability or alteration in arterial circulation to the kidney

4. A presumed disturbance of the venous return from the kidney

This investigation was undertaken to clarify certain points at issue particularly those regarding the incidence and pathogenesis of the postural varieties of benign proteinuria and their relationship to well established types of renal disease, particularly nephritis

Material and Methods

The investigations on the incidence of postural proteinuria and certain other studies were performed on healthy sea cadets aged 14 to 16 years, healthy medical students aged 20 to 30 years and on patients over the age of 50 years who were suffering from a variety of non-renal diseases

Certain patients with postural proteinuria discovered by army entrance examinations and others in hospital for various non-renal diseases were used for other aspects of the work   Where relevant, further details on the patients are included in the descriptions of the investigations

The following techniques were used —

Total serum proteins (Kagan (34))
Serum and urine albumin and globulin (Colorimetric) (Fine (17))
Urine proteins (Turbidimetric) (Daley (12))
Urea (Conway (11))
Diotrast clearance (Foa, Woods and Peet (20))
Diotrast estimation (Alpert (1))
Mannitol and Para amino hippurate clearance and estimation (Goldring and Chasis (23))
Thiosulphate clearance and estimation (Newman, Gilman and Philips (45))
Creatinine (Steinitz and Turkand (59))
Statistical methods (Fisher (19))
Cold pressor test (Hines and Brown (28))
Modified Addis' count and differential counts of urinary nucleated cells (Bull (10))

At certain stages of the work it was necessary to use the endogenous creatinine clearance as an index of the glomerular filtration rate because inulin and mannitol could not be obtained   Steinitz and Turkand (59) and Miller and Winkler (42) have shown that the endogenous creatinine and inulin clearances are almost identical in normal subjects   However, Smith, Finkelstein and Smith (57) point out that the endogenous creatinine clearance is always higher than the inulin clearance and in disease may be up to 42% higher   They state that this error is due to the presence of chromogens other than creatinine in the plasma   There is no reason to believe that

these chromogens should vary in their concentration over short periods so that while the absolute figures for the glomerular filtration rate by this method might be in error, comparison of the rates obtained in consecutive periods should provide a reliable index of altering glomerular filtration In the present study, only arguments based on altering rates were used and furthermore, the alterations found were often in excess of 42% For these reasons, the deductions drawn from the glomerular filtration rates obtained by using the endogenous creatinine clearance method are valid

RESULTS

I —*The Incidence of proteinuria and its relation to posture and age*

One hundred and twenty-nine healthy sea cadets, 50 healthy medical students and 50 ambulant male patients above the age of 50 years were examined as follows —

Specimens of urine were examined for protein by the turbidimetric method

a   Erect lordotic posture

b   Erect kyphotic posture

c   recumbent lordotic posture
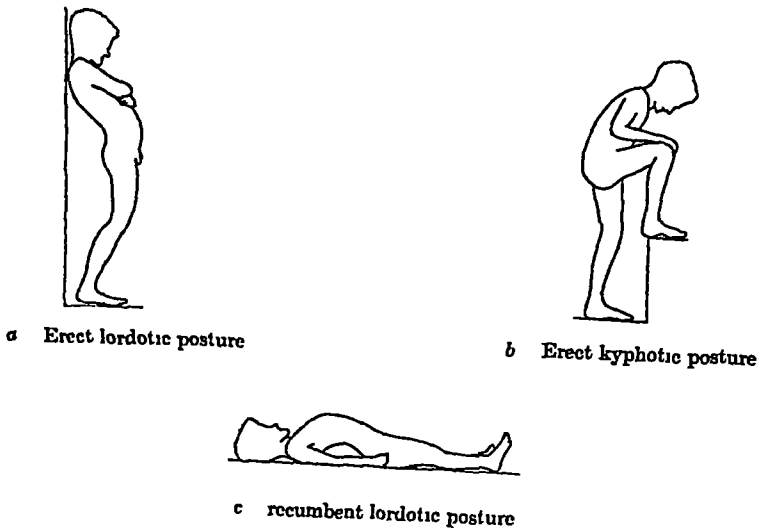
Fig 1

Urine specimen 1 was secreted during a period in which there had been no control of posture and during which no strenuous exercise had been undertaken

Urine specimen 2 was secreted during a period in which the subjects were made to stand immobile in a posture of maximum lordosis (Fig 1a) In the over 50 age group, the patients were seldom able to attain a significant degree of lordosis

TABLE I

The numbers and percentages of proteinuric subjects in different age groups and in different postures

Age group	Number of Subjects with proteinuria						
	Total No of Subjects	Specimen 1 Posture uncontrolled		Specimen 2 Erect lordotic		Specimen 3 Recumbent kyphotic	
14 16 yrs	129	60	46 5%	99	77%	0	0%
20 30 yrs	50	1	2 %	18	36%		
Over 50 yrs	50	1	2 %	6	12%		

TABLE II

The number of subjects of all ages divided into groups according to the amount of protein found in the urine

Age group	Protein conc	Specimen 1 Posture uncontrolled		Specimen 2 Erect lordotic		Specimen 3 Recumbent	
14 16 yrs (129 subjects)	0	≯68	52 7%	31	24 0%	99	100%
	Trace	30	23 3%	15	11 6%	0	0%
	+	14	10 8%	20	15 5%	0	0%
	++	10	7 7%	17	13 2%	0	0%
	+++	7	5 5%	27	21 0%	0	0%
	++++	0	0 %	19	14 7%	0	0%
20 30 yrs (50 subjects)	0	49	98 %	32	64 %		
	Trace	1	2 %	4	8 %		
	+	0	0 %	6	12 %		
	++	0	0 %	2	4 %		
	+++	0	0 %	5	10 %		
	++++	0	0 %	1	2 %		
Over 50 yrs (50 subjects)	0	48	96 %	44	88 %		
	Trace	1	2 %	4	8 %		
	+	1	2 %	1	2 %		
	++	0	0 %	1	2 %		
	+++	0	0 %	0	0 %		
	++++	0	0 %	0	0 %		

Trace — up to 9 mg /100 ml
+ — 10 to 29 mg /100 ml
++ — 30 to 99 mg /100 ml
+++ — 100 to 499 mg /100 ml
++++ — above 500 mg /100 ml

Urine specimen 3 was collected from those cadets who passed protein in urine specimens 1 and 2 and was secreted during a period in which they were lying in the recumbent kyphotic posture in hammocks

The results are shown in Tables I and II

Comment These data show that proteinuria was induced in the greatest number of subjects and in greatest concentration when the subjects were placed in a posture of extreme lordosis Table I shows that in all the subjects examined, no proteinuria occurred when they were in a recumbent kyphotic posture

The Tables also show that a variable and smaller percentage of subjects secreted protein when their posture was uncontrolled than when they were in a posture of erect lordosis Furthermore, the concentration of protein in the urine tended to be higher in erect lordosis and was very considerable in many cases, particularly in the younger subjects

It will be noted that approximately three-fourths of the youths, one-third of the young men and one-tenth of the men over the age of 50 had proteinuria

II —*The effect of posture and abdominal support on proteinuria*

For this study, 14 postural proteinuric subjects were used The tests were designed to analyse in greater detail the influence of various postures In addition, the effect of application of a tight abdominal binder on the proteinuria was tested The procedure was as follows —

After a period of uncontrolled posture, the subject was instructed to empty his bladder The urine was saved (specimen A) He was now placed in various postures in turn and at the end of each period in a new posture (usually about half-an-hour) he emptied his bladder and the urine was tested for protein by the turbidimetric method The following postures were used in the order listed —

Recumbent kyphosis (specimen B)

Recumbent lordosis produced by placing pillows under the lumbar spine (Fig 1c) (specimen C)

Erect kyphosis produced by standing with one foot on a chair (Fig 1b) (specimen D)

Erect lordosis with a firm abdominal binder applied The binder was a many tailed bandage tightened from below upwards and was applied with the subject recumbent (specimen E)

Erect lordosis without the abdominal binder (specimen F)

The results are shown in Table III

Comment The maximum proteinuria occurred when the subjects were in erect lordosis Furthermore, six of the subjects who secreted protein in erect lordosis also did so in the recumbent lordotic posture

TABLE III

The effect of posture and the application of a tight abdominal binder on postural proteinuria

Case No	Protein concentration in mg/100ml in each subject													
Posture	4	6	11	15	20	21	32	168	169	171	172	173	174	175
A Uncontrolled	0	0	3	40	5	10	0	0	100	0	0	10	0	0
B Recumbent kyphotic	0	0	0	5	0	0	0		0	0	0	0	0	0
C Recumbent lordotic	0	0	3	60	0	50	15		60	0	0	10	0	0
D Erect kyphotic	0	0	0	0	0	0	0		0	0	0	0	0	0
E Erect lordotic with binder	0	0	0	0	0	0	15	0	0	60	80	70	0	300
F Erect lordotic without binder	40	60	40	280	120	180	15	55	100	80	600	200	70	200

With the exception of Case 175, the proteinuria induced by the erect lordotic posture was lessened or abolished by the application of an abdominal binder   In Case 175 the application of a binder increased the proteinuria

With the exception of Case 15, all subjects secreted protein-free urine when in a kyphotic posture even when kyphosis was combined with standing Case 15, the only subject who secreted a small amount of protein in recumbent kyphosis probably did not empty the bladder completely before lying down, resulting in admixture of protein—containing (A) with the protein-free urine secreted in recumbent kyphosis   This was demonstrated to be the cause in several other instances in which the same phenomenon was found In every case, a further period in this posture caused the urine to become protein-free

It can be concluded from these observations that —

1   Postural proteinuria is maximal in erect lordosis but can be induced in some subjects in recumbent lordosis

2   The application of a firm abdominal binder usually prevents or diminishes the proteinuria induced by the erect lordotic posture with one exception only in this series

3   Proteinuria does not occur in recumbent or erect kyphosis

III —*The relationships of blood pressure, posture and proteinuria*

Amongst the current hypotheses of the pathogenesis of postural proteinuria are those relating the condition to vasomotor instability and renal disease   The following investigations were carried out to obtain evidence on the reactions of the circulation to the erect posture in proteinuric subjects as against "normal" controls   Healthy sea cadets were used and were divided into proteinuric (33 subjects) and non-proteinuric (14 subjects) groups as determined from Section I   Both groups were investigated as follows —

Fifteen mintues after lying down and while they remained supine, estimations of the blood pressure were made on the right arm Several readings were taken over the course of two minutes and further readings if the blood pressure continued to fall after $1\frac{1}{2}$ minutes If this occurred, estimations were continued until two readings were the same The lowest constant reading was recorded On completion of these readings, the subjects were made to stand up and 8 to 10 minutes later further recordings of the blood pressure were made by the same method

The results are shown in Table IV

TABLE IV

Blood pressure (averages) in postural proteinuric and non proteinuric subjects

	Blood pressure recumbent mm.Hg		Mean fall in pulse pressure on assuming erect posture mm.Hg	Mean rise of blood pressure on assuming erect posture mm.Hg	
	Systolic	Diastolic		Systolic	Diastolic
Proteinurics (33 subjects)	116 9	66	1 8	2 8	5 8
Non proteinurics (14 subjects)	110 5	66 8	0 4	3 6	4 6
Difference of means	6 4	0 8	1 4	0 8	1 2
Significance of difference of means	0 1⟩ P⟩0 05	0 8⟩ P⟩0 7	0 8⟩ P⟩0 7	0 9⟩P⟩0 8	0 8⟩P⟩0 7

Further evidence of differences in lability of the blood pressure between the postural proteinuric and non-proteinuric subjects was sought for by means of the cold pressor test The results are shown in Table V

TABLE V

The cold pressor test in proteinuric and non proteinuric subjects

(This table shows the mean maximum rise of systolic and diastolic blood pressures in the proteinuric and non proteinuric groups As the pressures in all cases returned to the basic level within 2 minutes of the application of the cold stimulus the time factor has not been analysed)

	Mean rise in blood pressure in mm.Hg	
	Systolic	Diastolic
Proteinurics (33 subjects)	5 7	10 7
Non proteinurics (14 subjects)	10 4	11 4
Differences of means	4 7	0 7
Significance of difference of means	0 1⟩P⟩0 05	0 9⟩P⟩0 8

Comment   It will be seen that there is no significant difference between the proteinuric and non-proteinuric subjects in respect of the characteristics examined

IV —*Correlation of proteinuria with body build*

The view has been expressed that postural proteinuria occurs more readily in persons of a certain build   The hypothesis was tested using 50 unselected sea cadets of age 14 to 16 years   The following data were obtained —

1   The concentration of protein in the urine secreted in erect lordosis

2   The age to the nearest month

3   The weight in pounds

_4   The height to the nearest half-inch

5   The ratio of weight/height

The weight/height ratio was used as an index of body build   In sthenic individuals the ratio should be high and in asthenics low

The correlation of proteinuria with the weight/height ratio was significant and suggested that proteinuria is commoner in subjects with high weight/height ratios, i e, sthenics   However, when this correlation was corrected for age it was not significant

None of the other correlations (Nos 1 and 2, 1 and 3 and 1 and 4) was significant   In view of the limited age group of 14 to 16 years used in this study, this does not mean that there might not have been a significant correlation of proteinuria with age if a wider age group had been used

Postural proteinuria is therefore, not related to height or weight and does not occur more commonly in sthenic or asthenic persons

The lumbar curves of the same subjects were examined and the degree of lumbar lordosis found to be very similar in the proteinuric and non-proteinuric subjects   This confirms an impression obtained from all the cases of postural proteinuria examined   Therefore, subjects with postural proteinuria do not have a tendency to undue lumbar lordosis

V —*The relationship of postural proteinuria to other medical conditions in a group of sea cadets*

To test the possibility that postural proteinuria is related to certain diseases, particularly nephritis, a medical history was taken from 46 unselected sea cadets and a routine clinical examination was carried out on the 129 unselected cadets used in Section I   Eighty-seven of these cadets were followed for 2 years and 42 for 6 months

a Past medical history The frequency of occurrence of certain illnesses is shown in Table VI

TABLE VI.

The frequency of occurrence of certain diseases from the past histories of proteinuric and non-proteinuric cadets

	Proteinuric group 32 subjects		Non proteinuric group 14 subjects	
	Number	%	Number	%
Chicken pox	21	65	10	71
Whooping cough	17	53	7	50
Measles	25	78	12	86
Mumps	10	31	7	50
' Tonsillitis ''*	13	40	7	50
German measles	3	9	1	7
Scarlet fever	3	9	2	14

* Under ' Tonsillitis '' are grouped all subjects giving a history of tonsillitis or who had had their tonsils removed

Osteitis, intussusception, enteric fever, broncho-pneumonia, malta fever, influenza, jaundice, migraine, asthma and diphtheria each occurred once in the proteinuric group Appendicitis occurred three times in the proteinuric group Malaria occurred twice and pneumonia and jaundice once each in the non-proteinuric group No case in either group gave a history of renal disease

b General examination and follow up study None of the cadets showed signs of œdema, cardiac enlargement or of arteriosclerotic or other changes in the fundi Two subjects had small varicoceles

The blood Wassermann reaction was done on 34 postural proteinuric and 16 non proteinuric cadets' sera and was negative in every instance The titre of cold agglutinins in the same sera was below 1 in 20 in every case The total protein content of the same sera was estimated and the mean value for the proteinuric and non-proteinuric groups was identical—7 7 gms per 100 ml

For the rest, apart from the proteinuria, general examination of all the cadets revealed no abnormalities

None of the cadets examined subsequently developed symptoms or signs suggestive of nephritis or other renal disease

Comment It will be noted that there is no disproportionate occurrence of any disease in either group Tonsillitis, which might be expected to predispose to nephritis, occurred less often in the proteinuric group than in the non-proteinuric but the difference is small and not significant Scarlet fever, which also predisposes to nephritis, occurred very infrequently in both groups and again there is no significant difference in incidence

Therefore, postural proteinuria can occur without a past history of renal disease or scarlet fever or tonsillitis which might predispose to renal

TABLE VII

Urinary sediment counts when the posture was uncontrolled

Protein conc	No of subjects	Formed elements in millions per 12 hrs					
		RBCs		Nucl cells		Casts*	
		Mean	Range	Mean	Range	Mean	Range
0	14	0	—	0 116	0—0 7	0 001	0—0 02
Trace	10	0 03	0—0 2	0 196	0—0 7	0 01	0—0 1
+	6	0 03	0—0 1	0 308	0—0 9	0 016	0—0 1
++	4	0 05	0—0 2	0 2	0—0 4	0 025	0—0 1
+++	8	0 43	0—1 6	1 44	0—3 1	0 002	0—0 3

TABLE VIII

Urinary sediment counts when in the erect lordotic posture

Protein conc	No of subjects	Formed elements in millions per 12 hrs					
		RBCs		Nucl cells		Casts*	
		Mean	Range	Mean	Range	Mean	Range
0	7	0	—	0 14	0—0 5	0	—
Trace	3	0	—	0 287	0 19—0 7	0	—
+	7	0	—	0 419	0—1 56	0 04	0—0 3
++	7	0 073	0—0 51	0 557	0—2 04	0	—
+++	8	0 41	0—2 6	1 066	0 2—5 2	0 35	0—2 6
++++	7	0 73	0—2 4	3 713	0—10 9	0 49	0—1 3

* All casts were hyaline or granular

Trace	1— 9 mg /100 ml
+	10— 29 mg /100 ml
++	30— 99 mg /100 ml
+++	100—499 mg /100 ml
++++	above 499 mg /100 ml

disease and there is no significant difference between proteinuric and non-proteinuric subjects in the incidence of past disease   Furthermore, none of the physical signs that characterise obvious nephritis accompany the proteinuria in postural proteinurics and a follow up study reveals no tendency for postural proteinurics to develop patent nephritis or other renal disease

VI —*Microscopic examination of the urine*

The rate of urinary excretion of cells and casts in subjects with and without postural proteinuria was determined in forty-two unselected sea cadets Urine specimen 1 which was secreted under uncontrolled conditions of posture and urine specimen 2, secreted in lordosis, were collected and examined microscopically   Total and differential counts were made on the sediment The results are shown in Tables VII and VIII

Differential urinary nucleated cell counts revealed no significantly different distribution of cell types in urine from postural proteinuric and non-proteinuric subjects

Comment   It will be seen that postural proteinuria is accompanied by an increase in the output of all formed elements in the urine

Differential nucleated cell counts show that the types and proportions of cells passed by postural proteinurics are similar to those found in non-proteinurics

VII —*Clearance studies*

This investigation was undertaken to establish the dynamics of kidney function in postural proteinurics   The renal clearances of various substances was investigated in different postures

The results are shown in Table IX and Fig 2 shows the results in a typical case in graphic form

Comment   It will be seen that there is little difference between the clearances in recumbent kyphosis and erect kyphosis but that there is often a considerable fall in the clearances of all substances in the erect lordotic posture

Table IX shows that the urine flow in the erect lordotic posture may drop to as little as 5% of that in the recumbent posture and that this drop in urine flow is usually considerably more than can be accounted for by the drop in glomerular filtration rate that accompanies it   The same phenomenon can be seen to have occurred in the urea clearance and indicates that both water and urea are being reabsorbed more in the erect lordotic posture than in the recumbent kyphotic posture

The effective renal plasma flow and the glomerular filtration rates are lower in the erect lordotic posture than in the recumbent kyphotic posture

Case No	Recumbent kyphotic							Erect kyphotic						
	Urine flow	Effective renal plasma flow	Glomerular filtration rate	Creatinine clearance	Filtration % fraction	Urea clearance	Chloride clearance	Urine flow	Effective renal plasma flow	Glomerular filtration rate	Creatinine clearance	Filtration % fraction	Urea clearance	Chloride clearance
308	23 8	798	115 5	'	14 5		2 34	18 0	620	122		19 7		3 08
310	2 36	850	126	139	14 9			3 42	440	148	150	33		
180	16 35	499		92	10 0*	70	1 7	16 15	490		103	21*	65 5	1 08
185	17 7	645		146	26 8*									
186	17 4	860				108		14 6	859				120	
309	2 33	323	113	179	35									
179	11 6 0 7			186 120				7 4 0 77			200 116			
199	7 9			210		193		1 95			125		116	
200	0 65			89										
171	7 1					64		3 3					45	
174	1 7					57								
187	4 1					68								

* Filtration fraction calculated from

but the fall in the former is greater than that in the latter resulting in higher filtration fractions in erect lordosis than in recumbent kyphosis In other words, a greater proportion of the plasma is filtered at the glomerulus in the former posture

VIII —*Protein secretion by each kidney*

This investigation was designed to determine whether one or both kidneys secrete protein during postural proteinuria Three subjects with this condition were used Mr Goldschmidt kindly inserted catheters into the ureters of these patients under local anaesthesia After urine flow had commenced through the catheters, the subjects were made to stand in forced lordosis and the urine secreted from the right and left kidneys collected separately and tested for protein

IX

Ml /Min

Case No	Erect lordotic							Clearances in erect lordosis divided by clearances in recumbent kyphosis expressed as a percentage						
	Urine flow	Effective renal plasma flow	Glomerular filtration rate	Creatinine clearance	Filtration % fraction	Urea clearance	Chloride clearance	Urine flow	Effective renal plasma flow	Glomerular filtration rate	Creatinine clearance	Filtration % fraction	Urea clearance	Chloride clearance
308	3 78	266	86 4		32 5		0 85	15 9	33 3	73		224		36
310	2 31	333	87 5	105	26 2			98	39 1	69	75	176		
180	2 09	395		77	23 6*	27	0 78	12 8	79		84	118*	38	46
185	11 0	306		98 5	32 2*			62	56		67	120*		
186	3 2	610				67		18 4	71				62	
300	1 13	259	102	110 6	39 5			48	80	90	65	113		
179	0 55 / 0 23			13 / 60				4 8 / 33				7 / 50		
190	0 68			156		140		8 6				74	73	
200	0 41			84				63				95		
171	0 55					35		7 8					55	
174	0 81					28		48					49	
187	1 3					25		32					37	

endogenous creatinine clearance

The results are shown in Table X   In Cases 164 and 187 it was fortunate that no blood contaminated the specimens but in Case 190, on assuming the erect posture, both catheters commenced to discharge slightly blood-stained urine   The urine from the right side contained more blood than that from the left

Comment   In Cases 164 and 187, the results clearly demonstrate that both kidneys secreted protein   In Case 190, owing to contamination of the urine with blood, one cannot be certain whether the right kidney secreted protein at all   One can, however, see that the left secreted protein because the urine from the left side contained more protein and less blood than that from the right

It may therefore be concluded that both kidneys may secrete protein in postural proteinuria
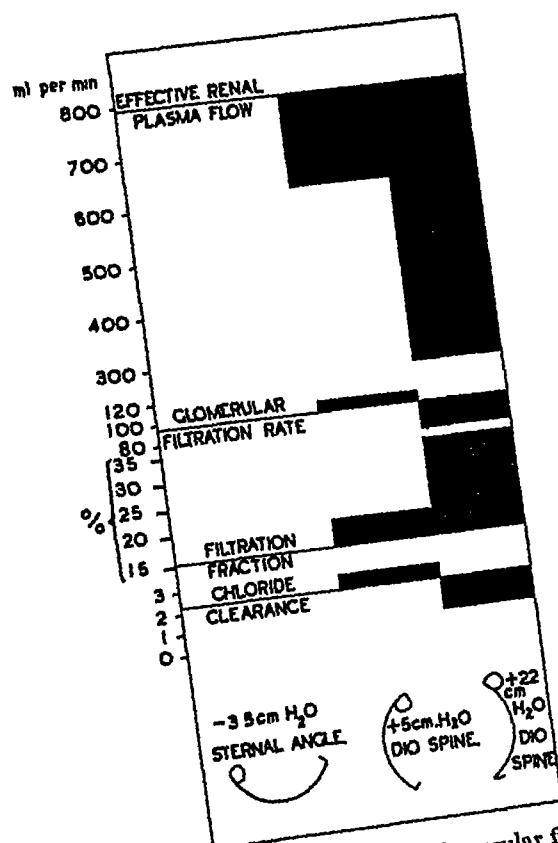
Fig 2   The effective renal plasma flow (ml per min), glomerular filtration rate (ml per min) chloride clearance (mg per min) and inferior vena cava pressure (c m H 0) in three postures in a subject with postural proteinuria

IX —Foot-to-tongue circulation times in proteinuric subjects and non-proteinuric controls

This investigation was designed to show any possible delay in venous return from the lower extremities which would indicate excessive delay in inferior vena cava circulation In preliminary trials, it was found that

TABLE X

Concentration of protein in urine from the right and left kidneys in postural proteinuric subjects

Case No	Right kidney, mg per 100 ml	Left kidney, mg per 100 ml
		87
	93	19
164	37	70
187	22	
190		

satisfactory end points were difficult to obtain when the subjects were in the erect posture, so subjects were chosen for this study on the basis of their having proteinuria in recumbent lordosis   Foot-to-tongue circulation times were determined using 10 ml decholin or 10 ml 10% calcium gluconate as the indicator substance   The results are shown in Tables XI and XII

TABLE XI

The foot to tongue circulation times in non proteinuric subjects in the recumbent kyphotic and recumbent lordotic postures

Case No	Recumbent kyphosis		Recumbent lordosis	
	Circ time, secs	Urinary protein, mg per 100 ml	Circ time, secs	Urinary protein, mg per 100 ml
159	31	0	27	0
160	24	0	27	0
161	8	0	9	0
162	10	0	11	0
163	9	0	8	0
165	19	0	23	0
166	35	0	39	0

TABLE XII

The foot to tongue circulation times in postural proteinurics in the recumbent kyphotic and the recumbent lordotic postures

Case No	Recumbent kyphosis		Recumbent lordosis	
	Circ time, secs	Urinary protein, mg per 100 ml	Circ time, secs	Urinary protein, mg per 100 ml
153	32	0	70	320
154	26	0	+	500
155	10	0	15	30
156	10	0	17	50
157	29	0	123	400
158	23	0	35	300

+ No end point

Comment It will be noted that in the proteinuric group, the foot-to tongue circulation times in recumbent lordosis are considerably greater than in recumbent kyphosis and that in this they differ from the non-proteinuric controls

TABLE XIII

Pressure readings in the inferior vena cava in two postures in subjects with postural proteinuria

Subject	Pressure in cms water from D 10 spinous process		Pressure rise cms water
	Erect kyphosis	Erect lordosis	
A.N	— 1	+ 19 5	20 5
A.M	+ 6	+ 18	12
S T	— 7	+ 10	17
B N	0	+ 14	14
C N	+ 5	+ 22	17
S G	— 4	+ 10 5	14 5
M S	+ 3	+ 17 5	14 5
G F	0	+ 12	12
S C	— 3 5	+ 17	20 5

TABLE XIV

Pressure readings in the inferior vena cava in two postures in subjects without postural proteinuria

Subject	Pressure in cms water from D 10 spinous process		Pressure rise cms water
	Erect kyphosis	Erect lordosis	
C P	+ 5 5	+ 15	9 5
W A	+ 4	+ 13	9
S W	+ 1	+ 9	8
C C	+ 4	+ 9	5
G C	+ 3	+ 11	8
G S	— 2	+ 6	8

The short circulation times obtained in Cases 155, 156, 161, 162 and 163 are explained by their youth (5 to 6 years) and small size, combined with fear of the test which increased the circulation rate

X —*Pressures in the inferior vena cava*

To obtain further evidence on the nature of the inferior vena caval circulatory delay suggested in the previous section, direct pressure readings were made in the inferior vena cava by means of a catheter The catheter was inserted by way of the basilic vein, subclavian vein, superior vena cava and right auricle and pressure readings were made on a water manometer using the spinous process of the 10th dorsal vetrebra as the point of reference Six subjects without and nine subjects with postural proteinuria were investigated The results are shown in Tables XIII and XIV

Comment It will be noted that a rise in pressure accompanies change of posture from erect kyphosis to erect lordosis and that this rise is significantly greater in subjects with postural proteinuria than in those without this condition

XI —*Anatomy of the inferior vena cava*

In view of the results of the two previous sections, it is obvious that there is a delay in the circulation in the inferior vena cava during the proteinuric phase To establish the cause and the site of the obstruction to the venous flow, anatomical dissections were undertaken

Twenty-eight adult cadavers were examined in the Anatomy Department of the University of Cape Town to determine the relationship of the liver to the upper part of the inferior vena cava In each case the liver was removed intact and that portion of the inferior vena cava which runs posterior to it was removed with it

Results It was noted that the inferior vena cava is surrounded to a variable degree by liver substance and that the length of the vessel in contact with the liver varied considerably Four specimens are shown in Figs 3, 4, 5 and 6 and were chosen to illustrate the great variability in the anatomy at this level

Comment In those subjects in whom the liver encases the inferior vena cava as in Figs 3 and 4, the intrahepatic portion of the vessel must follow the liver when this organ rotates In other subjects such as those shown in Figs 5 and 6 a wide range of rotation of the liver could not affect it Possibly more important from the point of view of compression of the inferior vena cava is the length of liver in contact with the vessel. The centre of rotation of the liver on a horizontal axis must be at or near the upper end of the groove for the inferior vena cava The greater the length

of posterior surface of the liver in contact with the inferior vena cava, the more likely is the latter to be compressed (*See* Fig 7, page 105)
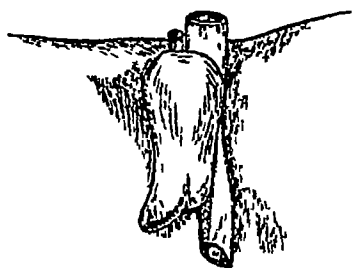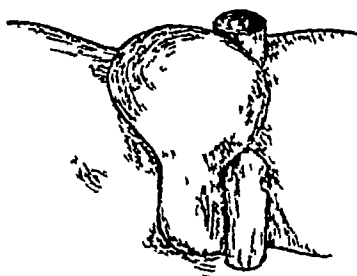
Fig 3   Fig 4
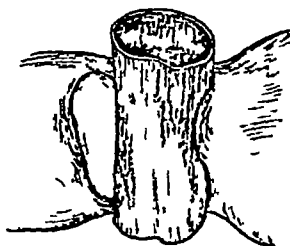
Fig 5   Fig 6

Figs 3, 4, 5 and 6   Posterior views of the liver to show its relation to the inferior vena cava

XII — *The effect of position of the liver on proteinuria*

This investigation was undertaken to determine whether proteinuria could be induced by rotating the liver into different positions Adult subjects were chosen for this investigation on the basis of their having easily palpable livers which could be manually rotated through the intact abdominal wall After a period of uncontrolled posture, the subjects emptied their bladders and the urine was saved (specimen A) Then the following manoeuvres were undertaken for 10 minutes each in the order listed At the end of each period a specimen of urine was collected and tested for protein

1   The subjects were placed in a posture of recumbent kyphosis (Urine specimen B)

2   They then remained in the same posture but manual pressure was applied to the liver in an attempt to rotate its anterior surface inferiorly (Urine specimen C)

3　They were then placed in a posture of recumbent lordosis and at the same time, the lower edge of the liver was pushed upwards causing the anterior part of the liver to rotate upwards　(Urine specimen D)

4　The liver was then released but the subjects remained in recumbent lordosis　(Urine specimen E)

5　Finally, pressure was applied to rotate the anterior surface of the liver inferiorly, the subject remaining in the posture of recumbent lordosis　(Urine specimen F)

After completion of these manœuvres, certain of them were repeated and foot to tongue circulation times done while the subjects were maintained in two of the positions

The results are shown in Table XV

TABLE XV

The effect of rotation of the liver on the urinary protein and the foot to tongue circulation time

	Uncontrolled posture	Recumbent kyphosis	Recumbent kyphosis plus inferior rotation liver	Recumbent lordosis plus upward rotation liver	Recumbent lordosis No manipulation of liver	Recumbent lordosis plus inferior rotation liver	Case No
	A	B	C	D	E	F	
Total protein mg /100ml	5	5	10	0	20	720	201
Globulin mg /100ml	0	0	0	0	0	80	
Total protein mg /100ml	0	—	—	0	10	330	repeat
Total protein mg /100ml	0	—	—	0	—	60	repeat
Circulation time secs				36		61	
Total protein mg /100ml	0	0	0	0	0	100	130
Total protein mg /100ml	0	0	0	0	0	10	131
Circulation time secs		33				†	
Total protein mg /100ml	0	0	0	0	10	30	132
Circulation time secs		22				51*	
Total protein mg /100ml	5	0	—	0	30	560	152
Total protein mg /100ml	0	0	—	—	—	300	repeat
Circulation time secs		17				65	
Total protein mg /100ml	10	10	—	10	10	30	151‡
Circulation time secs		35				130*	

† No end point

‡ The continuous proteinuria was caused by pyelitis

* At the times indicated both subjects tasted the Decholin　The liver was then released and some seconds later a much stronger taste was observed

Comment It will be noted that in each of the subjects, a position of recumbent lordosis with inferior rotation of the liver produced varying degrees of proteinuria In 3 of the subjects, recumbent lordosis alone was sufficient to induce the proteinuria and, in these, upward rotation of the liver abolished it

Comparison of the results of this investigation with those of Section II reveals a close similarity Here as in Section II, lordosis was a necessary factor but in most of the cases was insufficient by itself to induce the proteinuria When lordosis was combined with an inferior rotation of the liver, the proteinuria was maximal

It will be seen also that the same lengthening of the circulation time occurred in strict association with the proteinuria as was seen in Section IX This suggests that inferior vena cava compression was responsible

DISCUSSION

The type of proteinuria The proteinuric subjects in this study fall into the group of postural proteinurics The proteinuria could always be abolished by the assumption of a posture of kyphosis and induced by lordosis, either alone or combined with other factors

Exercise as a contributing factor was excluded in this series because the proteinuria occurred under conditions of rest or after mild exertion and because as Barach (3, 4), Hellebrandt (26) and Hellebrandt, Brogden and Kelso (27) showed, violent exercise is necessary to induce proteinuria

Neurological disease and renal palpation can also be excluded as causes

The various hypotheses of postural proteinuria listed on page 78 may now be considered seriatim

The hypothesis of an abnormality of the serum proteins

In 1905, Wright and Ross (69) found what they believed to be a hypocoagulability of the blood in two cases of " physiological albuminuria " They postulated that an increased hydrostatic pressure combined with the abnormality of the blood was responsible for the condition To test this hypothesis, they administered calcium salts to patients because of the then current belief that the coagulability of the blood could be increased by this means The albuminuria disappeared Fox (21, 22) confirmed the effect of calcium therapy

Post and Thomas (50) believed that it was not calcium as such that was responsible but that it acted by virtue of its basic properties They stated that they could regularly abolish the proteinuria by neutralisation or mild alkalisation of the urine

However, Wright and Ross, Fox and Post and Thomas do not appear to have realised the great importance of accurate control of posture in

any study of this condition   Nicholson (46) in a well controlled investigation was unable to confirm their work and Harrison (25) similarly found calcium to have no effect on the proteinuria   The suggestion that alkalisation of the urine abolishes the proteinuria was not substantiated in the present investigation   Although no deliberate attempt was made at investigating this point, several subjects spontaneously passed alkaline urine containing protein

A final decision on the question of the effect of calcium or alkali administration must rest on more complete study, but any effect that they may have must be of minor importance for circulatory factors must be present to account for the postural nature of the proteinuria

Hypotheses based on presumed congenital or acquired lesions of the kidney and particularly of the glomeruli

One cannot picture any alteration in the renal parenchyma which could occur with posture unless it were due to mechanical or reflex alterations in the blood supply to the organ as a whole   These will be considered later However, there remains the possibility that congenital or acquired lesions of the glomeruli do occur in postural proteinuria and cause them to be more permeable to protein   These lesions would allow them to pass protein under conditions of disturbed circulation which would not affect normal glomeruli

Congenital lesions   Among the earliest suggestions made to explain postural proteinuria were some that postulated congenital abnormalities of the glomeruli and associated abnormalities of vasomotor tone, build and habits   In conjunction with these, a " proteinuric diathesis " was described (14, 2, 6, 43)   Hooker (29) reviews these old hypotheses very fully

The present investigation reveals no special proteinuric diathesis and this finding is supported by Bashford (5) and Palmer (47)   However, Diehl and McKinley (13) investigating a large group of 16,748 students found postural proteinuria to be slightly commoner in people with a high height/weight ratio   The present smaller series of 50 subjects in whom the point was studied may not have revealed this slight correlation and it is possible that the condition is slightly more common in asthenic subjects

However, as postural proteinuria occurs in such a high percentage of people, one can hardly speak of an abnormality, congenital or otherwise Furthermore, congenital abnormalities do not tend to revert to normal with advancing age as would have to occur to explain the diminishing incidence in the older age groups   It might be suggested that the abnormal glomeruli become completely defunct with age but there is no histological evidence of such obliterated glomeruli in a sufficient proportion of normal adults' kidneys

Acquired lesions   Some schools of thought hold that postural proteinuria is a manifestation of nephritis or other renal disease and that it may be the

only evidence of such a lesion   Prominent among the upholders of this view are Russell (53,54) and Thorp and Wakefield (64)   Their main reason for this belief is their finding of cases with undeniable signs of nephritis plus postural increase of proteinuria   However, any condition which can affect three-fourths of the population can occur in a high percentage of people with other conditions including nephritis and these cases probably represent nephritics who have at the same time postural proteinuria   Russell also found a greater percentage of children who had recently had scarlet fever to have postural proteinuria than a control series   However, it appears from his papers that neither of these two groups of children was examined under uniform and controlled conditions of posture and exercise during the period of urine secretion   He believed that symptoms such as headache, fatiguability, lassitude and occasionally some swelling of the face were commoner in the proteinuric group than in normal subjects   This argument was, however, based on a highly selected series of cases referred to him and who must have had symptoms or else they would not have been discovered

Russell's and Thorp and Wakefield's conclusions are, therefore, not acceptable   Furthermore, on the evidence of the present investigation any causal relationship between postural proteinuria and nephritis is unlikely for the following reasons —

1   It is difficult to picture a type of nephritis that can cause such a characteristic and selectively postural proteinuria in about three-fourths of youths

2   If such a lesion exists, one would expect to find other evidence of it than the proteinuria   This is not the case

    a   No detectable œdema was found in any of the cases.

    b   The serum protein concentrations of the proteinuric and non-proteinuric groups of cadets did not differ in any way   This agrees with the findings of Schlutz and Swanson (56) and Linder, Lundsgaard and van Slyke (37)   If the proteinuric subjects had nephritis, one might have expected some lowering of their serum protein concentrations even although all readings were in the normal range

    c   None of the cadets showed evidence of arteriosclerosis on examination Only one of them had a raised blood pressure at any time   In this case the blood pressure in the erect posture was 165/95 mm Hg and when recumbent 125/70 mm Hg   The high reading was probably due to nervousness   Examined statistically there was no significant difference in the blood pressures in the proteinuric and non-proteinuric groups   Diehl and McKinlay (13) and Nicholson (46) came to the same conclusion   Had nephritis been responsible for the proteinuria, some difference in the blood pressures in the two groups might have been expected

d   None of the cadets with postural proteinuria gave a history suggestive of nephritis, œdema or any renal disturbances in the past

e   The histories of the cadets showed that there is no special incidence of diseases such as scarlet fever or tonsillitis which are liable to be complicated by nephritis   Furthermore, there was no disproportionate frequency of occurrence of the exanthemata in either the proteinuric or non-proteinuric groups

f   There was a lack of subsequent development of patent nephritis on follow up   Had the proteinuria been due to nephritis some cadets should have shown signs of it later

g   There is almost universal agreement that the prognosis for future life and health of postural proteinurics is excellent   This would not be the case if nephritis was responsible   Wolman (68) reviews this aspect very fully so that repetition is unnecessary

h   The nucleated cells in the urinary sediment are not of the same type as are found in nephritis or nephrosis (Section VI)   (The distribution of the various types of nucleated cells in urinary sediment in health and disease will be considered in a future paper)

Therefore, it can be concluded that nephritis plays no part in the pathogenesis of postural proteinuria   (In cases of acute diffuse glomerulonephritis in the stage of recovery an intermittent proteinuria is sometimes seen which may not be associated with posture   This group requires further investigation)

Hypotheses based on presumed disturbances of arterial circulation to the kidney

Among the earliest suggestions for the pathogenesis of postural proteinuria was one that vasomotor instability caused circulatory changes in the kidney and these in turn were responsible for the proteinuria (60, 14, 48)   The first experimental attempt at proving this hypothesis was that of Erlanger and Hooker (16)   They examined one subject with postural proteinuria and one control subject and noted that in the proteinuric, the proteinuria was accompanied by a fall in pulse pressure   Certain manœuvres such as immersion of the body in water or the application of a pneumatic suit, prevented both the proteinuria and the fall in pulse pressure   In three of their experiments (numbers 11, 59 and 67) the association of fall of pulse pressure and proteinuria did not occur   Later, Hooker, Hegemann and Zartman (31) published a brief report of another case in which there was the same association of a fall in pulse pressure with the proteinuria All these authors concluded that the fall in pulse pressure caused the proteinuria

Hooker (30) in certain perfusion experiments on isolated kidneys found that the amount of urine passed varied directly and the amount of protein

inversely with the magnitude of the pulse pressure   This was taken as further evidence favouring the hypothesis   Mason and Erickson (40) quoted further indirect evidence to show that renal metabolism was altered by lowered pulse pressure and described four cases of postural proteinuria in whom the proteinuria was accompanied by a fall in pulse pressure   As controls, they determined the blood pressures in 4 subjects without renal disease   In their subjects with postural proteinuria, the pulse pressures in the erect posture were 14, 32, 40 and 55 mm Hg   while in their controls 58, 49, 50 and 50 mm Hg   Despite the fact that all of these controls had lower pulse pressures than one of their postural proteinuries, their work has been extensively quoted in favour of the pulse pressure hypothesis   It appears that the 6 cases quoted form the basis on which the pulse pressure hypothesis has been carried through the literature (22, 50, 18, 70)

Investigation of the present larger series of cases revealed that the behaviour of the pulse pressure in postural proteinuries is the same as in non-proteinuries   On the assumption of the erect posture, proteinuria can occur whether the pulse pressure rises or falls   In support of the present finding is the work of Bass and Wessler (7) and Diehl and McKinlay (13)

The increased venous pressure hypothesis

The effect of increased venous pressure on the renal dynamics   Posnor (49) in 1880 induced proteinuria in animals by compressing the renal veins   This has been confirmed many times since then   Winton (66) working on dogs' heart-lung-kidney preparations induced proteinuria by raising the venous pressure and noted that accompanying the proteinuria was an oliguria and decrease in renal blood flow   He quoted certain histological evidence by Ludwig (39) to show that the rise in venous pressure dilated the peritubular venules and compressed the tubules with a consequent rise in intratubular pressure   This suggestion was confirmed by his work because he found that a rise in ureteric pressure which ordinarily reduced urine flow did not do so when the venous pressure was increased   This apparently paradoxical result is explained by the fact that the ureteric pressure is communicated to the tubules and prevents their compression by the dilated venules   Furthermore, when the ureteric pressure was raised first and the venous pressure later, it was possible to induce an increase in urine flow with the rising venous pressure   The interpretation of this was that the venous pressure was communicated back to the glomerulus and caused an increase in the filtration pressure

Theobald (63) working on dogs, showed that the normal pressure in the inferior vena cava was 4 to 8 mm Hg   and when this pressure was raised to 8 to 10 mm Hg , oliguria resulted   Between 10 and 15 mm Hg pressure, oliguria, albuminuria and retention of chloride, phosphorus and nitrogen occurred and between 15 and 20 mm Hg pressure, haematuria occurred as well   If the pressure rose above 20 mm Hg , anuria resulted

There is therefore, sufficient evidence that an increase in venous pressure will cause proteinuria and certain other manifestations in animals Theobald (62) was able to induce proteinuria in man by pressure on the epigastrium, presumably compressing the inferior vena cava   I have seen a case of Hodgkin's disease with gross proteinuria   At autopsy, both renal veins were surrounded by masses of glands and compressed   No renal lesion was found to account for the proteinuria so that venous compression by the glands was probably responsible   Cardiac failure is often accompanied by a raised venous pressure   Under these conditions there is frequently proteinuria and water and salt retention is a feature of the condition (65) The picture is, however, somewhat complicated and will be discussed in a future paper   Therefore, despite the lack of direct evidence, it is almost certain that an increased venous pressure does produce similar effects in man and animals

By inference from Winton's work, we should expect to find a rise in the filtration fraction because the venous pressure is probably transmitted to the glomerulus and tends to raise the filtration pressure   This rise in the filtration fraction is not likely to be large because of the parallel increase in intratubular pressure   The effect of the increased intratubular pressure and decreased renal blood flow on the glomerular filtration rate would therefore only partly be compensated by the rise in filtration fraction and a decrease in glomerular filtration rate should result

Therefore, if postural proteinuria is due to an increased venous pressure mechanism we should find —

1   Proteinuria
2   Oliguria
3   Diminution of chloride excretion
4   Diminution of urea excretion
5   Hæmaturia if the rise in venous pressure is sufficient
6   Decreased renal blood flow
7   Increase in the filtration fraction
8   Lowering of the glomerular filtration rate

1   *Proteinuria* of course occurs by definition

2   *Oliguria*   Section VII shows that there is a decreased urine flow during the stage of proteinuria and that the fall may be considerable   This feature has been noted before (38)

3   *Diminution in chloride excretion*   Section VII shows that the clearance of chloride may fall considerably with the occurrence of proteinuria   This has been noted before to be a regular feature of postural proteinuria (16, 38)

4   *Diminution in urea excretion*   Section VII demonstrates lowered urea clearances accompanying the proteinuria   This has been noted previously (16, 55)

5   *Haematuria*   Section VI demonstrates that there is an increased passage of erythrocytes in the urine and that this parallels the degree of proteinuria   Rytand (55) also found an increase in the numbers of red cells in the urine of 5 patients during the phase of proteinuria

6   *The renal blood flow*   Section VII shows that there is a decrease in the effective renal plasma flow accompanying the proteinuria

7   *The filtration fraction*   Section VII demonstrates that there is a rise in the filtration fraction accompanying the proteinuria

8   *The glomerular filtration rate*   Section VII demonstrates that there is a fall in glomerular filtration rate accompanying the proteinuria This change has been noted before and shown to be a characteristic feature of postural proteinuria (41)

Therefore, the renal dynamics in postural proteinuria are consistent with the hypothesis that the proteinuria is due to an increase in venous pressure

Possible causes of increased venous pressure

Three possible mechanisms might give rise to increased pressure in the renal veins

1   A general increase in venous pressure

2   The renal veins may be compressed unilaterally or bilaterally

3   The inferior vena cava may be compressed

1   *A general increase in venous pressure*   This possibility can be ruled out immediately because there is no distension of the veins of the neck or other evidence of congestive cardiac failure in postural proteinurics

2   *Compression of the renal veins*

a   *Left renal vein compression*   Kelling (35) suggested that compression of the left renal vein by the superior mesenteric artery was responsible for postural proteinuria   This would explain why lordosis alone can produce proteinuria in some individuals and why lordosis plus the erect posture does so even more effectively   The erect posture, by causing visceroptosis, would close the angle between the aorta and the superior mesenteric artery Support for Kelling's suggestion came from Sonne (58) who catheterised the ureters of 6 subjects and found that the urine from the right ureter contained no protein while on the left there was anuria or proteinuria   One of his subjects had bilateral anuria   Rieser and Rieser (52) found that the

application of an abdominal binder, correcting visceroptosis, prevented the proteinuria and this was taken as further evidence favouring the theory that the "aortamesenteric pincers" obstructed the left renal vein The present study confirms Rieser and Rieser's facts (Section II) Finally, Rytand (55) showed radiologically that an increase in the size of the left kidney occurred during the period of proteinuria in two out of 5 subjects This suggested that it was distended by the increase in venous pressure on that side

However, there are certain objections to this hypothesis of left venal vein compression —

(1) The proteinuria may be bilateral Table 16 shows the results of ureteric catheterisation of subjects with postural proteinuria derived from the literature and from Section VIII

TABLE XVI

Protein content of the urine from the right and left kidneys in postural proteinuria

Author	No of cases	Protein in the urine	
		Right kidney	Left kidney
Sonne (58)	1	Anuria	Anuria
	3	0	Anuria
	1	0	+
	1	0	++++
Beer (9)	2	0	+
Prince (51)	1	‡	+
	1		+ Trace
Theobald (62)	4	+	+
Elkhorn (15)	1	+	0
Section VIII	1	‡+	++
	1	+	++
	1		+

It will be noted that 8 of the 18 cases passed protein bilaterally and a ninth had right and left sided anuria One case had unilateral right sided proteinuria

(2) The renal functions are altered to a degree greater than would be expected from a unilateral disturbance Section VII shows that when the subject is in an erect lordotic posture, the urine flow, urea clearance and creatinine clearance may drop to levels considerably below 50% of those in the kyphotic posture If only the left kidney was affected, these functions should not be depressed by more than half

(3) The foot-to-tongue circulation time is prolonged   (Section IX)
This cannot be explained by a renal vein mechanism and suggests
inferior vena cava compression

(4) The pressure in the inferior vena cava is raised   (Section X)

Because of these facts, the left renal vein hypothesis does not explain
the whole picture

b Double renal vein compression   There is no obvious explanation
on an anatomical basis to explain why the application of a binder to the
abdomen should prevent compression of the right renal vein   This vein
takes a short and direct course from the inferior vena cava to the kidney
and is not enclosed in a pincer as is the left   A neural reflex from the left
kidney to the right in response to the left sided venous distension might be
postulated but is difficult to prove   However, this hypothesis can also be
excluded on the basis of the prolonged foot-to-tongue circulation time and
the raised pressure in the inferior vena cava

3   *Inferior vena cava compression*   Jehle (33) suggested that the upper
lumbar spine in lordosis kinked and compressed the upper part of the inferior
vena cava   This explains very well the fact that lordosis causes proteinuria
and overcomes the objections to the renal vein hypothesis   The erect
posture favours lordosis and this explains why it can induce proteinuria
It does not explain, however, why the proteinuria in the erect posture is
greater than in the recumbent lordotic posture (Section II) nor why the
application of a binder to the abdomen abolishes the proteinuria

All the facts are best explained on the basis of a new hypothesis which
will now be presented

The liver and inferior vena cava hypothesis of postural proteinuria

It is suggested that the mechanism of postural proteinuria is as follows
When the subject is in a kyphotic posture, the distance between the
diaphragmatic opening of the inferior vena cava and its origin at the junction
of the common iliac veins is least and the inferior vena cava is relatively lax
If the subject is now placed in a lordotic posture the vein is stretched and
the part behind the liver is brought into close apposition with the spine
Now the upper end of the inferior vena cava is surrounded to a variable
degree by liver substance (Section XI) and any rotation of the liver about
the axis of its area of fixation to the posterior part of the diaphragm will
tend to rotate the inferior vena cava with it and compress it against the
spine   The degree of rotation and the extent of compression will depend
on its relation and degree of fixation to the inferior vena cava   Some
rotation of the liver may occur in recumbent lordosis because its anterior
aspect is less fixed than its posterior, but the erect posture will favour this
rotation much more because the liver's centre of gravity lies in front of the
centre of rotation   This hypothesis is illustrated in Fig 7
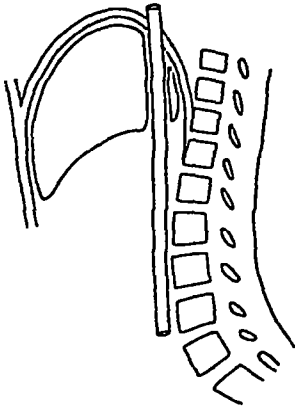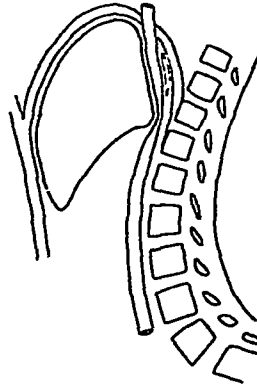
<center>Fig 7a   Fig 7b</center>

Fig 7a   The relations of the liver inferior vena cava and spine in an erect slightly kyphotic posture

  b   The relations of the liver inferior vena cava and spine in the erect lordotic posture

This hypothesis explains why lordosis is necessary for the production of proteinuria and why erect lordosis is more effective than recumbent lordosis   It explains bilateral proteinuria and the considerable lowering of renal function that can occur   The fact that one kidney may secrete more protein than the other is explained by local differences of venous return from the two sides such as might be caused by the " aortamesenteric pincers "

The hypothesis also explains the increased pressure in the inferior vena cava, the increased foot-to-tongue circulation time and the response to the application of an abdominal binder   If the binder supports and elevates the liver, preventing its inferior rotation proteinuria will not result even in the erect lordotic posture, but, if it catches the lower end of the liver and rotates it inferiorly it may increase the proteinuria   In Case 175 in Section II the liver was probably caught under the binder   The great anatomical variations in the relations of the inferior vena cava to the liver explain why subjects respond to posture with varying degrees of proteinuria

Immobility in lordosis is more effective in inducing proteinuria than is lordosis with movement (32) because it is not possible to maintain continuously the same degree of lordosis when moving

Further evidence favouring this hypothesis was obtained in Section XII by study of patients whose livers could be artificially rotated   It will be noted that these subjects were postural proteinurics in that certain postures were necessary for the production of proteinuria   In some cases it could be induced by lordosis alone and increased by further manual rotation of the liver   Furthermore, where proteinuria occurred spontaneously, upward

rotation of the liver entirely removed it The same delay in the foot-to tongue circulation time occurred as had been found in the postural proteinurics These cases, therefore, only differed fiom the usual postural proteinurics in having livers that could be artificially rotated

The variation in incidence of postural proteinuria with age requires further study However, it is probable that diminished mobility of the spine in older people or a change in the rate of growth of the spine and liver with consequent alterations of their relationships to the inferior vena cava account for the phenomenon

Summary and Conclusions

1 A survey of past work on postural proteinuria reveals considerable difference of opinion regarding the incidence and pathogenesis of the condition The hypotheses advanced to explain the proteinuria have been divided into the following groups —

a The suggestion that there is an abnormality of the blood

b The presumption that there are congenital or acquired lesions of the kidney and particularly of the glomeruli

c Presumed vasomotor instability or alteration in arterial circulation to the kidney

d A presumed disturbance of the venous return from the kidney

2 Investigations designed to throw light on these hypotheses and on the incidence of the condition have been presented and reveal —

a Postural proteinuria can be induced in approximately three-fourths of youths, one-third of young men and one-tenth of old men

b Postural proteinuria is maximal in erect lordosis but can be induced in some subjects in recumbent lordosis It does not occur in recumbent or erect kyphosis The application of a firm abdominal binder usually prevents or diminishes the proteinuria induced by the lordotic posture

c There is no significant difference between postural proteinuric and non-proteinuric subjects in respect of resting blood pressure or changes in blood pressure in response to posture or the cold pressor test

d Postural proteinuria is not correlated with body build

e Subjects with and without postural proteinuria show no difference in the incidence of past disease and none of the physical signs that characterise nephritis accompany the proteinuria Follow up study reveals no tendency for postural proteinuric subjects to develop patent nephritis or other renal disease

f Postural proteinuria is accompanied by an increase in the output of all formed elements in the urine

g Clearance studies reveal a very characteristic pattern of renal dynamics Accompanying the proteinuria there is a fall in effective renal blood flow and glomerular filtration rate and a rise in the filtration fraction

h Protein can be found in the urine from both kidneys

i A delay in foot-to-tongue circulation time was found in subjects with postural proteinuria in strict association with the proteinuria

j The pressure in the inferior vena cava is higher in the erect lordotic than in the erect kyphotic posture and the rise in pressure is greatest in subjects with postural proteinuria

l Considerable variation in the relations of the inferior vena cava to the liver were demonstrated

l Proteinuria was induced in subjects with large, easily palpable livers by rotating the liver inferiorly

3 In view of these findings, current hypotheses are inadequate to explain postural proteinuria It is suggested that the mechanism is as follows —

A rise in pressure in the inferior vena cava is produced by compression of the vessel against the spine by the posterior surface of the liver and this pressure is conducted back to the kidney inducing passive congestion and proteinuria The compression only occurs when the subject is in a lordotic posture and when the anterior surface of the liver rotates inferiorly This rotation of the liver normally occurs when the subject is lordotic and is maximal in the erect lordotic posture

Since completion of this paper further investigation has revealed that in some if not all subjects the site of compression of the inferior vena cava may be at the diaphragmatic opening and not below it as was suggested The conclusion that position of the liver and a lordotic posture combine to produce the effect remains unaltered.

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DESOXYCORTICOSTERONE-LIKE ACTIVITY INDUCED
BY ADRENOCORTICOTROPHIN IN MAN

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EVIDENCE for increased activity of all the known functions of the human adrenal cortex in response to stimulation by adrenocorticotrophin was presented by Thorn and his colleagues in 1947 (33)   In considering the control of the secretion of the adrenal cortex by the anterior pituitary gland, it has become apparent that there may be species differences in the nature of the response to anterior pituitary stimulation   It is probably possible from urine analysis to obtain a fair idea of " 11-oxysteroid " activity of the adrenal cortex and a more accurate one of androgenic activity from the excretion of the neutral 17-ketosteroids, approximately two-thirds of the latter being normally derived from the adrenal cortex   On the other hand, this is not true of those hormones showing desoxycorticosterone-like effects, which are confined to regulation of electrolyte metabolism (32)   For this reason it has become important, in assessing the degree of such activity of the adrenal cortex produced by adrenocorticotrophin, to study carefully evidence of change in electrolyte metabolism   Consideration of this phase of activity forms the subject of the present communication   A detailed investigation of other phases of adrenal cortical activity under the influence of adrenocorticotrophin is fully reported in a separate paper (14)

Methods

Urine specimens were collected at 12-hourly intervals and preserved with a few drops of chloroform-thymol preservative and stored in the refrigerator

Blood samples were collected on the morning of each day in the fasting state

* Rockefeller Travelling Fellow in Medicine

Stool specimens were collected in three-day periods and stored in the refrigerator   Uniform sampling for analysis was aided by the use of a Waring blender

Diet samples for analysis were obtained by setting aside aliquots of the daily rations

The following methods of analysis were used —

Sodium in serum, urine and diet, Consolazio and Dill (4),

Potassium, Consolazio and Talbott (5),

Chloride in serum and urine, Schales and Schales (29), with the precautions advised by Asper, Schales and Schales (1),

Phosphate in urine, Fiske and Subbarrow (13),

Carbon dioxide combining power of plasma, Peters and Van Slyke (27),

Total nitrogen in serum and urine by the micro Kjeldahl modification of Keys (19),

Total nitrogen in stools and diet by a modification of the macro Kjeldahl method due to Van Slyke (34),

Non protein nitrogen in blood by a modification of the method of Daly (7)

The patient (M R) considered in the present study, was a 46-year old male suffering from a mild degree of hypopituitarism (14)   He had been given, one year previously, X-ray therapy to a chromophobe adenoma of the anterior pituitary which had produced some reduction of pituitary function with slight secondary reduction of adreno-cortical function   This patient was chosen as a subject with the expectation that he would show the maximum increase in adrenal cortical activity in response to adrenocorticotrophin

He was maintained in a metabolism ward with thermostatic temperature control during the month of February, and was given a constant diet rich in cereals, fruit and milk with 1 5 g of added salt per diem   The diet pro-

TABLE I

Alterations in Serum Composition

Day	Period	Milliequivalents per litre of serum water *				Protein g per 100 ml serum	Haematocrit per cent
		Na	K	Cl	CO.		
3	Control	153	5 7	117	26	7 61	38
4	ACTH (1)	142	5 1	107	23	6 95	36
6	ACTH (1)	147	5 5	111	30	6 87	32
9	ACTH (2)	153	5 2	110	31	6 65	33
12	Control	156	5 3	110	34	6 30	34

* Calculated using the formula $W_s = 990 - 0 8 P$, where W is ml serum water per litre of serum and P is serum protein g per litre serum (35)
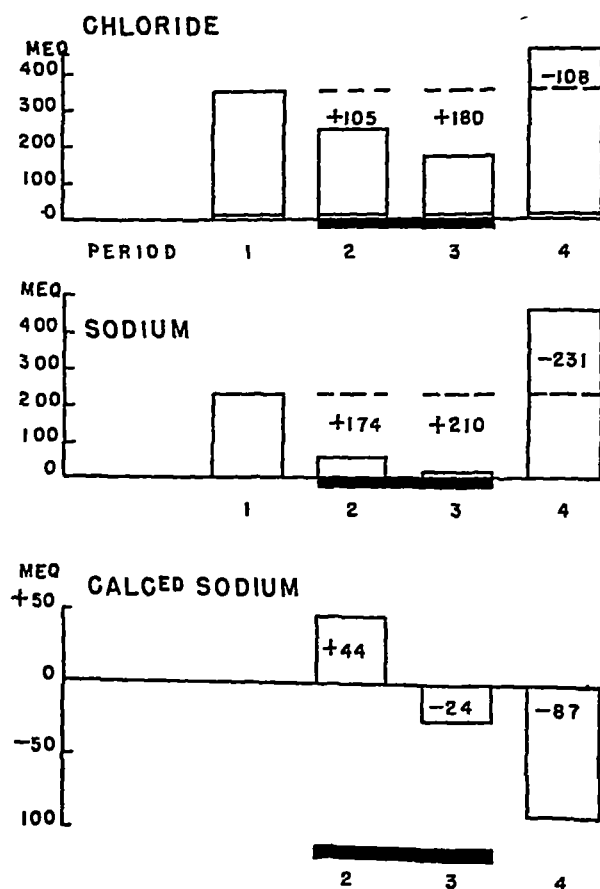
Fig 1 The chloride sodium and calculated sodium balances The double line at the base of the chloride balance indicates fæcal excretion (6 MEq) In the balance charts figures with + sign indicate MEq retained, with — sign, MEq lost Period 1 represents 3 day control period periods 2 and 3 3-day periods of ACTH administration in dosage of 40 mg per day in divided doses and period 4 is a 3-day after period Figures with + sign in the calculated sodium balance indicate sodium retained in excess of that calculated from the chloride balance (see text) and figures with — sign indicate the amount by which the excretion exceeded the calculated excretion

vided 1745 calories, 5 9 g nitrogen,* 74 milliequivalents sodium,* 94 milliequivalents chloride,* and 58 milliequivalents potassium† daily After a six day preliminary period to attain equilibrium he was observed during a three day control period He was then given 40 mg adrenocorticotrophin (14) daily in doses of 10 mg six-hourly for a period of six days This was followed by a second three-day control period

* Figures by analysis
† Calculated from Sherman 6th ed

Results

(a) *Changes in serum composition*

The data on changes in serum composition are given in Table I   All constituents showed an initial decrease associated with sudden hæmodilution Thereafter sodium and carbon dioxide combining power increased, whereas potassium and chloride remained at a constant level

(b) *Changes in Chloride and sodium balances*

The fluctuations in chloride balance are depicted in Fig 1 in which the sodium balance is also represented   The fæcal chloride excretion remained constant at 6 milliequivalents per three-day period

From the chloride balance was calculated the sodium balance Assuming that chloride did not enter the cells to an appreciable extent the " calculated sodium balance " should represent the sodium theoretically retained in extracellular fluid (8)   The ratio of sodium chloride in the extracellular fluid was determined (Table II) from the calculated concentration of extracellular sodium and chloride, using the Donnan factors of 1 04 for the ratio of serum sodium to extracellular fluid sodium and 0 97 for serum chloride to extracellular fluid chloride (16)   The observed changes in chloride balance were multiplied by these ratios and hence the " calculated sodium balance " obtained

Fig 1 shows that during the first period of administration of adrenocorticotrophin there was a very large retention of sodium and chloride, the former exceeding the calculated amount by 44 milliequivalents   During the second three-day period the observed sodium retention was less than that calculated by 24 milliequivalents   At this time the three-day output of sodium was only 6 milliequivalents, and renal retention was therefore at a maximum   Chloride retention was not, however, maximal   In the " after period " sodium was lost in excess of calculated sodium to the extent of 87 milliequivalents

(c) *Potassium balance*

During the first three days of adrenocorticotrophin administration, compared with the control period, there was a net potassium loss of 105 milliequivalents   This diminished to 6 milliequivalents during the subsequent three days   In the " after control period " 60 milliequivalents were retained, leaving a net deficit of 51 milliequivalents during the whole experiment (Fig 2)

(d) *Fluid balance*

During the control period the fluid intake was 2,500 ml per day, during the administration of adrenocorticotrophin 1,920 ml per day, and in the " after control period " 2,500 ml per day   These variations in intake were

POTASSIUM

NITROGEN

INORGANIC PHOSPHORUS
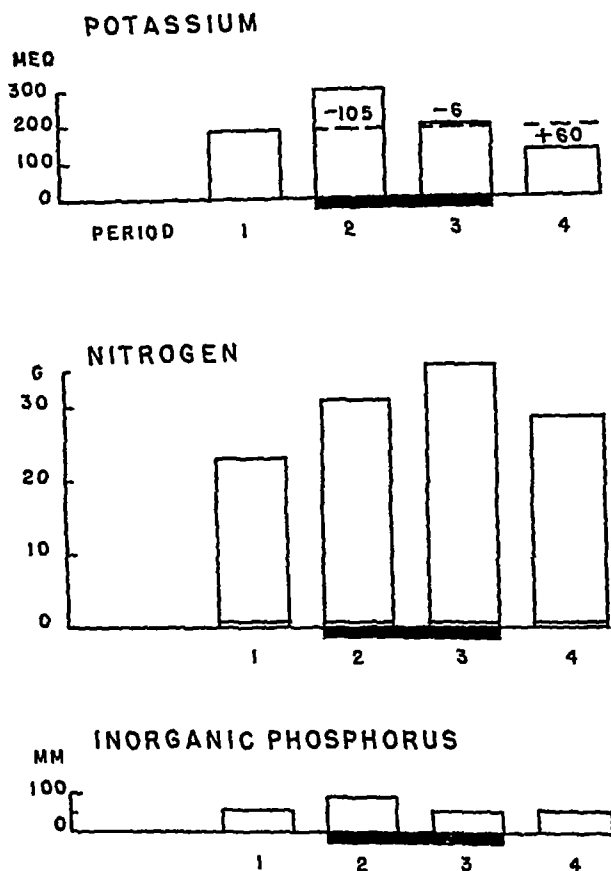
Fig 2 The potassium and nitrogen balances and urine inorganic phosphorus excretion Notations as in Fig 1

taken into account in assessing the balance (Fig 3), but insensible and faecal water losses were assumed to be constant (24) There is fair agreement between overall water gain and loss, and weight variation During the first three-day period of adrenocorticotrophin administration there was a retention of 1,040 ml of water, and 775 ml during the second three-day period In the "after control period" 2,965 ml of water were lost, representing a net deficit of 1,150 ml of water

In the lower part of Fig 3 are depicted the changes in extracellular fluid volume calculated from the chloride balance In the first three-day period of adrenocorticotrophin administration chloride retention amounted to 105 milliequivalents (Fig 1) and extracellular fluid concentration was 114 milliequivalents (Table II) The chloride retention indicated the expansion of extracellular fluid volume to be 922 ml Observed water

WATER BALANCE

WEIGHT KG 62 3   64 I   61 3

EXTRACELLULAR FLUID
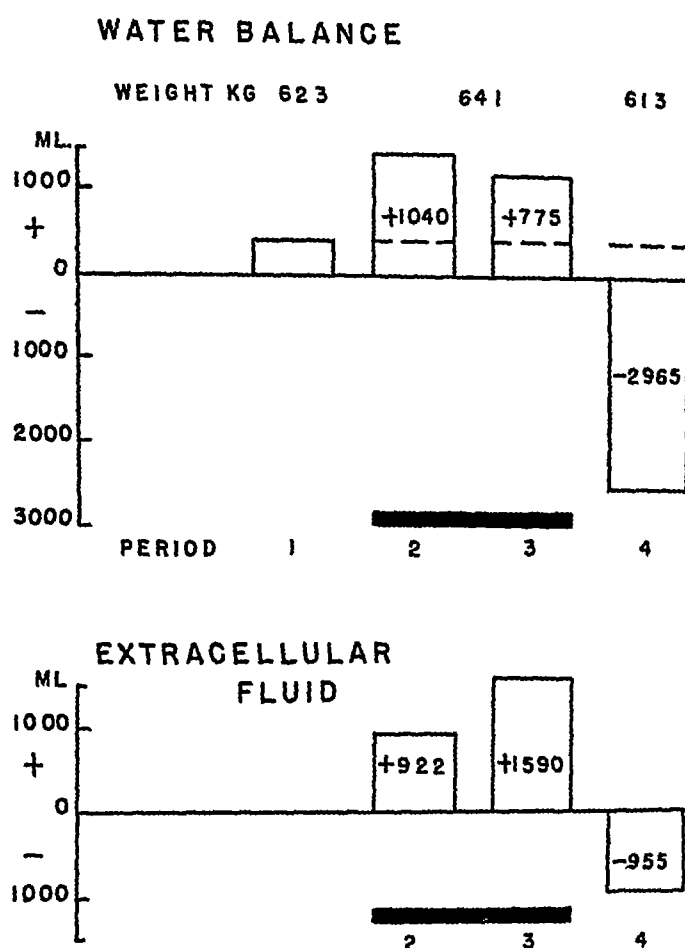
Fig 3   Water balance and calculated changes in extracellular fluid volume — *(see text)* In the lower diagram figures with + sign indicate the expansion of extracellular fluid volume in the corresponding period and figures with — sign the amount of decrease in this volume

TABLE II

Extracellular fluid composition

Calculated from serum concentrations *Table I, using Donnan factor of* 0 97 *for chloride and* 1 04 *for sodium*

Day	Period	Milliequivalents per litre		$\frac{Sodium}{Chloride}$ Ratio
		Sodium	Chloride	
3	Control	147	121	1 21
6	ACTH (1)	141	114	1 24
9	ACTH (2)	147	113	1 30
12	Control	160	113	1 33

retention was 1,040 ml and hence this water was almost completely retained in the extracellular fluid compartment Similarly during the second three-day period of adrenocorticotrophin administration the 775 ml of retained fluid were kept in the extracellular fluid compartment and an additional 815 ml transferred to it from the intracellular fluid In the "after control period" the extracellular fluid contributed 955 ml· to the 2,965 ml lost and the intracellular fluid 2,010 ml

The effect of adrenocorticotrophin therefore was initially to expand the extracellular volume by one litre by water retention Subsequently it was still further increased by one-and-a-half litres by both water retention and transfer of intracellular water After stopping the adrenocorticotrophin the net change was a transfer of seven-tenths of a litre of intracellular water to extracellular fluid and a further loss of two litres of intracellular water It will be noted the changes in extracellular fluid volume followed those in the hæmatocrit

(e) *Intracellular changes*

The data presented lend themselves to a computation of the order of magnitude of the corresponding intracellular fluctuations The calculated changes are tabulated in Table III It was assumed that the subject

TABLE III

Calculated alterations in intracellular composition

I lectrolytes expressed in MM per 100 g fat free solids water in g A 62 Kg man assumed to have 5 700 g muscle solids *

Before treatment	MM	ACTH 1st period ± MM	± %	ACTH 2nd period ± MM	± %	After period ± MM	± %†
Sodium	8	+ 0 8	+ 10	+ 0 4	+ 5	— 1 1	— 14
Potassium	48	— 1 9	— 4	— 2 2	— 5	— 1 1	— 2
Sodium plus Potassium	56	— 1 1	— 2	— 1 8	— 3	— 2 2	— 4
Water	345 g	+ 2 g	+ 0 6	— 12 g	— 3	— 47 g	— 14

* Data from Darrow (8)
† Cumulative changes from the initial state

possessed 5,700 g fat-free muscle solids, that the muscles represented the bulk of the body tissue in which ionic alteration was taking place and that the initial muscle composition was that represented in the first column (8) Sodium retained in excess of that calculated from the chloride balance (Fig 1) was considered to have become intracellular The deficit of sodium

over the calculated retention on the other hand, represents intracellular decrease In a similar way potassium excretion (Fig 2) represents intracellular potassium loss and potassium retention intracellular gain The figures for change in intracellular water are calculated from the balance depicted in Fig 3 In computing the intracellular potassium changes allowance was made for alterations of total potassium in the extracellular compartment due to fluctuations in the volume of the latter

During the first three days of adrenocorticotrophin administration there was 10% increase of intracellular sodium, and 4% decrease of potassium During the second three days the muscle sodium increased no further, due doubtless to the fact that maximum sodium retention was already taking place (Fig 1) and potassium loss increased slightly, with the small loss of intracellular fluid already noted In the " after control period " the sodium loss exceeded the previous retention, the net overall loss amounting to 14% About half the potassium was recovered, but the net change of sodium plus potassium was a decrease of 4% from the original Simultaneously a large intracellular water loss occurred

Discussion

Derivation of changes in the composition of extracellular and intracellular fluids from balance experiments involves the assumption that chloride remains entirely extracellular in its distribution This assumption is, so far as present evidence goes, a valid one, although small amounts of chloride may cross the cell membrane (8), (36)

Sodium on the other hand diffuses from one compartment to the other and hence, given the extracellular fluid concentrations of these ions and their balance, shifts of sodium may be calculated

Potassium is primarily intracellular but diffusion may readily occur The question of potassium loss associated with protein breakdown needs consideration Nitrogen, potassium, and phosphorus occur in protoplasm in definite proportions, and catabolism of protoplasm involves excretion of these substances in their relative proportions (28) Protein breakdown associated with adrenal cortical steroid activity is known to be associated with gluconeogenesis and hepatic glycogen formation The authors' own observations have suggested that the latter process is quantitively greater than the former Further, the formation of a large amount of hepatic glycogen has been induced in the patient under discussion by administration of adrenocorticotrophin (14) Such glycogen deposition is associated with intracellular potassium uptake (28) The data (Fig 2) show that in this case the excretion of potassium and phosphorus were maximal when nitrogen excretion was still low Therefore a renal effect rather than a protoplasmic catabolic effect would account better for the observed changes which resemble those produced by desoxycorticosterone Moreover we have obtained evidence (14) that potassium excretion of similar magnitude, under like

conditions, can occur in the normal individual in the absence of a negative nitrogen balance   Hence in these circumstances loss of potassium due to breakdown of protein has been neglected

Analysis of changes in serum electrolytes (Table I) demonstrates that there is a rise in the ionic strength of the extracellular compartment towards the end of adrenocorticotrophin administration and in the " after control period "   This was mainly due to increases of sodium and of carbon dioxide combining power, a change noted to take place in some cases of Cushings' syndrome (23), (3)

The adrenocorticotrophin used in this experiment contained 0 12 units oxytoxic activity per mg   and hence the patient received a total of 4 8 units per diem   The possible role of the small pituitrin contaminant was controlled in the patient at a later time, after sodium and chloride retention had been produced by a smaller dose of the same adrenocorticotrophin (2 5 mg , 6 hourly for 3 days)   Following this period, 1 unit of pituitrin was given 6 hourly for 3 days, that is nearly four times the contaminating pituitrin present in the adrenocorticotrophin   In the pituitrin period, compared with the adrenocorticotrophin period, there was a marked rebound rise in sodium and chloride excretion   The authors have confirmed this lack of pituitrin effect compared with that of adrenocorticotrophin in a normal subject and in addition observed that a patient with Addison's disease showed no evidence of fluid, sodium or chloride retention when given this adrenocorticotrophin (14)   These observations are in agreement also with the small changes observed in a normal subject receiving 20 units of pituitrin per day (30) Hence, although it is still possible that this posterior pituitary contamination may have had a part in the production of the water retention observed, the distribution of the water retained would appear to be a function of the desoxycorticosterone-like activity induced by the adrenocorticotrophin The likely sequence of events is probably as follows   The hormone administration provoked water, sodium and chloride retention by the kidney to a marked degree   In the initial stages water retention predominated, but not in the later stages   This latter event led to a rise in extracellular fluid osmotic pressure, with the result that on withdrawal of the hormone and a sudden release of water by the kidney, which again appeared to be the primary event, a large part of the excreted fluid was diverted from the intracellular fluid to restore osmotic relations between the extracellular and intracellular compartment   The situation is thought to be analogous to that encountered by McCance (22) where administration of sodium chloride to dehydrated individuals increased the fluid output, presumably at the expense of intracellular fluid   He also observed (2) that during dehydration and rehydration there was a strong tendency for the maintenance of extra-cellular fluid volume and ionic concentration by means of fluid losses from the cells with a rise in intracellular osmotic pressure   In fact, under similar conditions to those under discussion there was a predilection for intracellular potassium and water loss, with a net rise of intracellular osmotic pressure,

rather than the excretion of extracellular sodium and chloride to restore osmotic balance   It would appear therefore that the first step in the readjustment to basal conditions in our patient was mainly a function of changes in the cells secondary to alterations of renal function   This is also in accord with the findings of others (10), (15)

In our experiment intracellular water loss exceeded decrease of ionic concentration   This has often been noted to occur (8), and ability to alter the osmotic activity of intracellular compounds must be postulated (6)

The magnitude of the observed increase of intracellular sodium under the influence of adrenocorticotrophin may be compared with the data of Darrow and Miller (9) in rats   These authors employed relatively enormous doses of desoxycorticosterone and produced alterations about four times as great as those observed here, but otherwise similar   Expressed in millimols the potassium loss was about twice the sodium gain in each case   The magnitude of this change is further emphasised by comparison with changes of similar size, but in the opposite sense after total adrenalectomy   Thus Conway and Hingerty (6) observed, five days after adrenalectomy, a decrease of sodium of 3 millimols and a potassium increase of 8 millimols per 100 g muscle solids

It may be concluded then that administration of a preparation of adrenocorticotrophin in the human can produce an effect similar to known actions of desoxycorticosterone (31), (32)   These changes are (*a*) water retention which is mainly extracellular , (*b*) sodium and chloride retention, accompanied by increase of intracellular sodium, and rise of extracellular sodium concentration and carbon dioxide combining power , (*c*) intracellular loss of potassium which in this case was probably minimised by a concurrent glycogen deposition

This effect on electrolytes should be contrasted with the effects of " 11-17-oxy-steroids " which promote excretion of both sodium and potassium and those of androgenic adrenal hormones which promote a slow retention of both of these cations (14)

The conclusions drawn from the experiment in man discussed above are at variance with results derived from observations in the rat   There is strong evidence to suggest that the response of the rat adrenal cortex to the pituitary is significantly different   It has been found that hypophysectomy leads to adrenal atrophy, which affects primarily the zona fasciculata, and apparently although ketosteroids disappear from the fasciculata they persist in the glomerulosa (12)   Using the same preparation of adrenocorticotrophin as that employed by the authors, Deane and Bergner (11) obtained histochemical evidence of increased production of ketosteroids in the fasciculata, but not the glomerulosa   The glomerulosa was thought to produce desoxycorticosterone-like hormones   Further, Ingle and his colleagues (18) have observed, using Evans' preparation of adrenocorticotrophin, that in the rat there is a slight tendency to sodium and chloride loss rather than retention   In periods of short duration (four

hours) the authors have observed similar-salt diuresis in man (14)   This
may be due to predominant " 11-oxysteroid " activity during this time
In the rat it would also seem that this type of activity predominates, the
secondary role of the desoxycorticosterone-like activity being in accord
with the well-known fact that the adrenalectomised rat can be maintained
on salt with relative ease, as contrasted with the human with Addison's
disease or after bilateral adrenalectomy (17)

Mason and his co-workers (21) using Evans' preparation of adrenocorti-
cotrophin, were unable to demonstrate sodium and chloride retention in
man   Although the adrenocorticotrophic activity of our material assayed
in hypophysectomised rats is similar to that of Evans, the two preparations
are not identical, being obtained by different methods of preparation from
the pig and sheep respectively   Furthermore the preparation of Evans is an
electrophoretic entity, whereas our preparation exhibited two components
(20)   More important than differences in the properties of adrenocortico-
trophin preparation used however, is probably choice of subject   In the
present experiment the subject was deliberately chosen in the hope that the
maximum effects of adrenocorticotrophin would be observed

It has been suggested that in man anterior pituitary insufficiency is
clinically associated with greater manifestations of hypoglycaemia than of
deficient electrolyte metabolism, whereas the reverse is true of Addison's
disease   In the former state adrenal cortical deficiency is a secondary
atrophy, whereas in the latter it is primary and the whole cortex is usually
involved   However, more careful investigation is yielding information
that in hypopituitarism salt metabolism is impaired (25)   In our patient
the Kepler water excretion test was strongly positive   The observations
of McQuarrie quoted above (23) also tend to indicate that stimulation of
desoxycorticosterone-like activity may occur as a result of increased pituitary
activity

SUMMARY

1   A male individual with mild hypopituitarism was given 40 mg
adrenocorticotrophic hormone daily for six days

2   Large amounts of water, sodium and chloride were retained, with
an increase of intracellular sodium

3   There was potassium loss in the urine, with a decrease of intra-
cellular potassium

4   Extracellular fluid volume was increased and maintained for a
period after withdrawal of the hormone at the expense of intracellular fluid

5   It is concluded that the adrenocorticotrophic hormone is able to
stimulate desoxycorticosterone-like activity of the adrenal cortex under
favourable circumstances

6   The significance of these findings is discussed, and a probable species difference between the rat and man is indicated

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THE INVESTIGATION AND DIAGNOSIS OF STEATORRHŒA

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FAT excretion in disease must be considered in relation to the normal physiology of fæcal fats Bloor has reviewed this subject recently (6), and only certain points need be recalled here The quantitative study of fæcal fat excretion in the human subject began in Carl Voit's physiological laboratory in Munich For many years during the latter part of the last century his assistants were engaged in investigating the origin of fæcal fats and measuring the utilization of dietary fat, as part of more general studies of the physiology of nutrition Müller (19) demonstrated the continued excretion of fats in the fæces of two professional fasters, Cetti and Breithaupt Subjects kept on a fat-free diet of meat showed a similar fat excretion (18) and Müller concluded that the normal fæcal fat was not simply a food residue but was to a great extent secreted by the intestine Rubner (25), studying the utilization of fat taken in different forms and in different quantities, found that with intakes up to 214 g the daily output of fæcal fat did not vary greatly, ranging from 2 5 to 8 2 g Only with higher intakes was a large increase noted in the amount of fæcal fat, but even on a diet containing 351 g of butter daily, 87% of the ingested fat was absorbed An exception was noted in the case of bacon fat ("Speck"), when an intake of 96 g resulted in a fæcal output of 17 2 of fat daily Rubner had to use short (2-3 day) periods of study because the monotonous and abnormal diets he used soon produced nausea, but he achieved accurate results by very painstaking separation of the fæces of the experimental period In demarcating the experimental period he made use of the fact that a milk diet produces white fæces, a meat diet black fæces, so that he used one of these diets to show the beginning and end of his experimental periods He found that with moderate intakes, the amount of fat absorbed is independent of the type of fat the amount of roughage in the diet and the protein and carbohydrate intake, and, as may be seen by comparing the fat outputs found with fat-containing and fat-free diets, dietary fat is almost completely utilized This is true of most naturally occurring food fats, but

fats with a melting point above body temperature, such as mutton fat (m p 49° C) may not be completely utilized, and Arnschink (1) showed that stearin (m p 60° C) is very poorly utilized—only 9% was absorbed in the dog It has since been found that, while the amount of fat excreted on fat-containing and fat-free diets is not greatly different, fat-containing diets do produce some increase in faecal lipids (30) especially with high fat intakes (33), and even on fat-free diets more fat is excreted than in starvation

Although an endogenous origin was accepted for the greater part of the faecal fats, their source remained controversial Desquamated epithelium, bile, bacteria and intestinal secretions were recognised as possible sources Burger and Oeter (7) found that there was not enough sterol in the intestinal mucosa to account for the amount in the faeces Sperry (28) confirmed this and also showed (27) that, after excluding bile from the intestine by means of a fistula, the faecal fats increased, though the animals were on a fat-free diet Bacterial synthesis of faecal lipids has not been excluded Sperry and Angevine (29) thought it could not account for more than 40% of the faecal lipids They kept dogs with ileal and colonic fistulae on a fat-free diet and estimated the fat excreted from the small and large intestine separately They were able to determine that more lipids were excreted from the small intestine than had previously been found in intact animals' faeces, while from the blind colonic stump they were able to collect less than one-fifth of the amount of the fat previously excreted in the faeces They concluded that lipid is secreted into the small intestine , a large part of this secretion is reabsorbed, apparently in the large intestine The remainder, with a small amount secreted into the colon, makes up the greater part of the endogenous lipid found in the faeces

The composition of the normal faecal fats has also been studied Munk (20) found that the melting point of faecal fat varied according to whether lard (m p 35-37° C) or mutton (m p 50-53° C) was given With the lard the melting point of the faecal fat was 43-46° C, with the less well utilized mutton it was nearer that of the food, 51-54° C In general, faecal fat has a melting point 4-8° C higher than food fat (13) Sperry and Bloor (30) found that while the quantity of faecal lipids was largely independent of the amount eaten the nature of the excreted fat was influenced by the type of fat eaten, and resembled the blood fats, from which the faecal fats were probably derived Up to 40% of the faecal fat is unsaponifiable (26) and this fraction consists mainly of sterols (15) These are made up of about 50% coprosterol and the rest cholesterol (8) from which coprosterol may be derived The melting point of coprosterol is high (94 5° C) and this partly accounts for the high melting point of faecal fats compared to dietary fats A high proportion of the saponifiable fat occurs in the " split " form, mostly as soaps, partly as free fatty acids (26) Splitting of fat occurs readily in faeces (31) and does not depend on the presence of pancreatic lipase (21)

The accurate investigation of fat excretion is of interest in the study of mild and recovering cases of steatorrhœa and in the investigation of obscure anæmias Few such investigations have been made, for they have to be carried out over a relatively long period of time to minimize the error of fœcal collection because of the difficulty, noted by Rubner, of separating off the fæces belonging to a given dietary period The only convenient method of demarcating fæcal collections is with markers such as carmine or charcoal These are not absorbed and colour the fæces, but a single marker given by mouth becomes distributed over two or three stools as fæces are mixed in the colon For this reason balance studies, in which the output of a substance is compared with the intake over a given period, have to be carried out over a long period, at least twelve days (22) unless, as in the case of nitrogen balance studies, most of the substance is excreted in the urine and the error in fæcal estimations is negligible in relation to the total excretion Most quantitative studies of fat absorption (9) (35) have been carried out over too short a period to be accurate

Rokers, Pack and Rhoads (24) studied two subjects over 48 and 70 days and found the daily fæcal fat excretion to be 2 1 g in one subject on an intake of 75 g daily, and 3 0 g in the other, on an intake of 60 g daily Fat excretion was studied by a 12-day balance technique by Bassett and others (2) in idiopathic steatorrhœa and by Black and others (5) in tropical sprue The present work consists of an application of a 12-day balance technique to the study of fat excretion in 6 normal subjects, 4 patients with steatorrhœa and 3 other patients In the normal subjects, and in some of the patients, fæcal nitrogen losses were also determined

METHODS

Each set of observations was made over a twelve-day balance period, divided into three 4-day periods Except where otherwise noted, the diet contained 70 g of fat and 70 g of protein daily, as calculated from food tables (McCance and Widdowson, 1946) The calorie value of the diet ranged from 2200 cal to 2500 cal in different subjects The higher intake was found to be not quite sufficient for the normal subjects who were doing a day's work in the department, but patients in bed preferred the lower intake A 70 g fat intake was chosen as sufficiently high to measure normal fat absorption but not so high as to upset patients with mild steatorrhœa The observations on the six normals were not made concurrently but on one or two subjects at a time

The balance technique was as follows Before the beginning of the 12-day collection period, the patient was given the balance diet for three days On the evening of the third day the patient swallowed a carmine marker (0 6 g) and stools up to and including the appearance of the first marker were discarded At the time the first marker appeared the rectum was washed out, using about 500 ml of normal saline, and the result of the

washout was also discarded The 12-day collection began with the next stool passed, and was made in three consecutive periods, each corresponding to 4 days' dietary intake Stools were marked off by the carmine markers given at the end of each 4-day period , when the marker appeared in the stool the collection for that 4-day period was completed by a rectal washout of normal saline In spite of the use of markers and washouts, regular collection was dependent on at least one daily bowel movement, and if constipation was noted during the preliminary 3-day period on diet, vegetable mucilage (Isogel), 2-3 drams, was given daily No aperients were given since diarrhœa may in itself affect fat absorption Rectal washouts, suggested by Rekers, Abels and Rhoads (23), are not usually necessary for complete fæcal collections in most normal people, but they were done as a routine because we found they improved the accuracy of fæcal collection in patients with steatorrhœa, in whom rectal emptying is often incomplete Stools were transferred from bedpans to porcelain casseroles, in which the total 4-day collections were weighed and mixed The transfer from the bedpans involved a small loss of fæces As the normal subjects did not use bedpans but used the casseroles for defæcation, no such error was involved in the normal collections

The fat was estimated gravimetrically after Soxhlet extraction with petroleum ether of an aliquot portion of the wet fæces, previously treated with HCl and dried with plaster of Paris , split fat was estimated by titration with alcoholic NaOH (11) Soaps were not separately estimated In calculating the weight of split fat, a molecular weight of 268 was assumed for the mixed fæcal fats (12) In some patients the split fæcal fat was found to be 100% or more of the total fæcal fat, and it is clear that this assumed value for the molecular weight of the fæcal fats may occasionally be too high Fæcal nitrogens were estimated by the Kjeldahl method

RESULTS

Balances in normals

The results of the fæcal fat analyses in the six normal subjects give a mean daily fat excretion of 2 88 g which agrees with the results of previous workers (Table I) Since the fæcal fat may be an intestinal secretion and not unabsorbed dietary fat, the results have not been reported as a percentage of dietary fat An analysis of the 4-day fat excretions shows that these are variable, not only from subject to subject but for each subject *There are* no significant differences between the subjects The standard deviation of the 4-day fat excretion is found to be 3 83 after eliminating differences between subjects Since the mean is 11 54 g , the coefficient of variation is 33% The cause of this large variation in 4-day fæcal fat figures is probably two-fold It partly reflects collection errors due to the difficulty of accurately demarcating fæcal collection periods There may also be a true variation

TABLE I

Fæcal fat and nitrogen losses in six normal subjects on a daily intake of 70 g fat and 70 g protein

Subject	Weight of 4-day collection (g)	Weight of dried fæces (g)	Fat as percentage of dried fæces	Total fat as split fat %	Total fat excreted in 4 days (g)	Daily fæcal nitrogen (g)	Fat nitrogen ratio
W ♂	497	137	58	85	8·0	18	44
	274	110	89	65	98	125	78
	502	104	123	64	128	12	107
R ♂	615	102	143	74	146	16	91
	388	97	99	84	96	155	62
	550	137	83	65	113	18	63
F ♂	516	77·5	120	77	93	14	66
	880	176	895	57	157	25	68
	305	76	93	63	71	13	55
I ♀	376	125	160	62	200	16	125
	337	101	177	43	179	155	115
	283	56·5	160	47	91	078	117
Q ♀	550	96	112	92	107	12	89
	542	95	50	77	475	18	76
	523	118	112	35	132	16	82
S ♀	315	71	128	68	91	16	57
	270	112	109	77	122	18	68
	500	159	785	42	125	22	38

Analysis of fat excretion figures, to show variation not due to differences in the subjects

	Degrees of freedom	Sum of Squares	Mean Square	SD
Subjects	5	70 75	14 15	
Error	12	175 83	14 65	3 83
Total	17	246 58	14 5	3 81

Mean 4 day fat excretion = 11 54

Standard error of mean 4-day fat excretion $= \sqrt{\dfrac{14\ 65}{18}} = 0\ 902$

Standard deviation of sum of any three 4-day periods =

$$3\ 81 \times \sqrt{3} = 6\ 53$$

in fat excretion in normal subjects on a constant fat intake   The variation in the fat-nitrogen ratios in the 4-day periods could be explained in the latter way, for collection errors would affect fat and nitrogen excretion similarly and the ratio might be expected to vary little if the true fat and nitrogen excretions were constant on the constant diet   The practical conclusion to be drawn from the wide variation in 4-day fat collections is

that small differences in 4-day fat figures have to be interpreted cautiously and small increases in fat excretion cannot be detected using a short balance period   With these reservations, the probable upper limit of normal for a single 4-day fat estimation may be taken as 23 g   (mean + standard deviation × 3) for this series of observations   To detect small increases in faecal fat excretion, the mean of several 4-day excretions may be compared with the mean normal excretion, assessing the significance of the difference of means by " Student's " *t* test, or it may be more convenient to use an estimate of the maximum normal fat excretion for three 4-day periods   The present data give a probable upper limit of 54 2 g   of fat for 12 days (12-day mean plus 3 times its standard deviation), but it should be noted that this small number of observations gives only an approximate estimate of the standard deviation and therefore an unreliable estimate of the normal limits   With these data, the *t* test is a more reliable test of significance

The percentage of the total fat occurring as split fat in these people varied widely, from 35% to 92%, and it is clearly fallacious to regard a percentage splitting higher than 75% as abnormal (14)   It should be remembered, however, that the stools were 4-day collections, and though they were stored in a refrigerator during the collection period some splitting might be expected to have gone on while they were stored (3)

The faecal nitrogen (Table I) varied between 3 12 g and 10 g for the 4-day periods (mean 6 34, standard error of the mean 0 30, standard deviation 1 56)   In subsequent tables the faecal nitrogen excretions are expressed as mean daily excretions and for the normal subjects the range of the mean daily excretion was 0 8-2 2 g

Balances in patients with steatorrhœa

The variability of fat excretion in patients with steatorrhœa was assessed in the same way as for the normals, using the observations on the effects of treatment which are reported below   Nine 12-day balance periods from three patients were available for analysis (Table II)   The fat intake of the patients varied more than in the normals, by up to 20% of the mean intake for the 12-day period, and for this variation a correction has been made as shown in the table   This correction introduces an error which is probably negligible compared with the variation in fat excretion found   The results of the analysis show that in patients, as in normals, the coefficient of variation of single 4-day fat excretions is about 30%   With so great a variation, it is clear that when fat absorption in steatorrhœa is investigated by the balance technique, apparent changes in the amount of fat excreted have to be interpreted with caution   The relative value of 4-day and 12-day collection periods in the investigation of steatorrhœa was studied in three patients (Table III)   Two had idiopathic steatorrhœa and one had tropical sprue

TABLE II

Patients with steatorrhœa

Four-day fat excretion figures corrected for variations in fat intake during 12-day observation periods

Patient	12-day period	Average daily fat intake, g	Corrected 4-day excretion figures* g		
A (R I No 03465/46)	1	50	114	93	96
	2	74	107	49	50
	3	67	31	57	29
F (R I No 06991/47)	1	62	39	38	32
	2	88	39	65	52
	3	85	76	92	61
	4	57	35	64	40
D (R I No 70551/47)	1	68	37	57	83
	2	70	62	47	47

* Corrected 4 day fat excretion =

$$\text{observed 4-day excretion} \times \frac{\text{mean intake of three 4-day periods}}{\text{recorded intake of the period.}}$$

Analysis of variance to show variation in 4-day collections

	Degrees of freedom	Sum of squares	Mean square	Standard deviation
Subjects	8	9990	1248 5	—
Error	18	5493	305 2	17 47
Total	26	15483	595 5	

Mean 4-day fat excretion, 58 96

These subjects are not a homogeneous group as was the case with the normals

Patient F was a housewife of 41 with idiopathic steatorrhœa who had suffered from anæmia and attacks of diarrhœa since early adult life   Her symptoms had become severe in recent months when she had noticed her stools were pale and she was losing weight

Patient D, also a woman with idiopathic steatorrhœa, was a school teacher aged 38 who had had flatulence and diarrhœa for eight years and

who had developed symptoms of osteomalacia and severe anæmia during the two years before admission

Patient A was a monk of 75 who had done missionary work in Poona from 1930 to 1931, and then returned to this country   He had had severe symptoms of steatorrhœa for five months and had not responded to dietary treatment   He was regarded as an example of tropical sprue with a long period of latency

TABLE III

Effect of treatment on fat absorption

Patient	Date	Treatment	Mean daily fat intake, g	4 day fat excretions g			Mean 12-day fat absorption %
A	30 11 46	None	50	102	88	110	50
	12 12 46	Folic acid	58	105	—	73	61
	7 1 47	Folic acid	74	105	50	52	77
	20 4 47	Liver and yeast	67	31	56	29	85
	15 9 47	Folic acid (5 months)	70	12 day excretion	564 g *		93
F	22 1 47	None	62	38	38	33	85 5
	1 3 47	None	88	39	66	50	85 5
	19 3 47	Folic acid	85	77	93	60	77
	3 8 47	Folic acid (5 months)	57	38	59	39	79
D	13 4 47	None	68	38	58	78	80
	7 9 47	Folic acid (5 months)	70	62	47	47	81

* Four day demarcation of periods not possible owing to constipation

After a preliminary estimation of fat absorption without treatment, folic acid (Lederle "Folvite", 20 mg daily) was given   Fat absorption was estimated again at intervals during treatment   The two patients with idiopathic steatorrhœa showed no significant change in fat absorption after five months' treatment   In the patient with tropical sprue absorption improved steadily with treatment (Table III and Fig 1)   The mean fat absorption as estimated over three 4-day periods before folic acid treatment was 49 3%   Unfortunately, a stool was lost during the second 12-day period   As estimated over five 4-day periods (not consecutive) after

beginning folic acid treatment the mean fat absorption was 70 6% and the difference between the two means was significant   Treatment with folic acid was interrupted in this patient and replaced for three months with liver and yeast extract   A further improvement in fat absorption apparently occurred from 77% to 86%, but the difference was not statistically significant   With folic acid treatment for a further five months, fat excretion finally dropped to 56 4 g in 12 days, which is only slightly more than the probable normal maximum   The very gradual change in fat
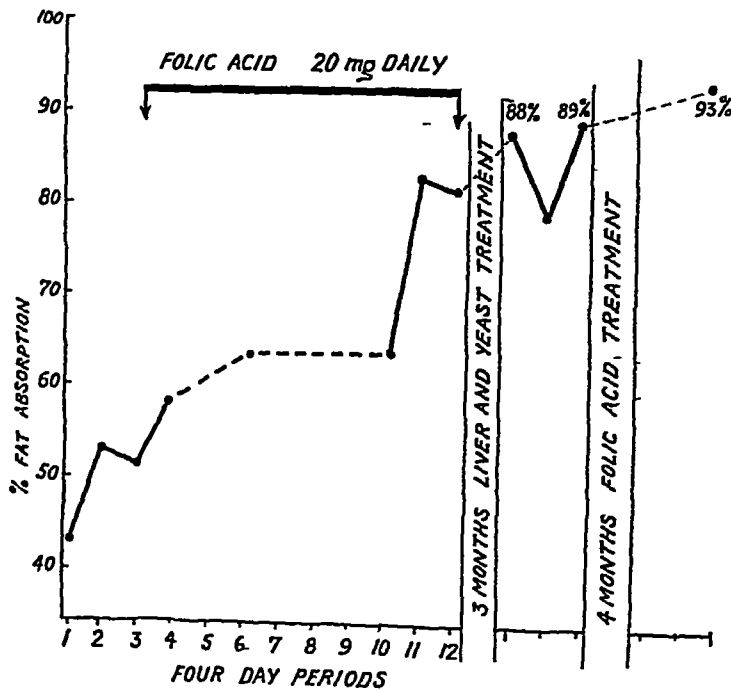
Fig 1   Fat absorption determined over successive 4-day balance periods in a patient with tropical sprue treated with folic acid

absorption contrasted with the dramatic effect of folic acid on the patient's sense of well being, appetite and diarrhœa, which was evident within two days

In one patient (F) data were obtained on the effect of change in fat intake on fat absorption (Table III)   In the first 12-day period the fat intake averaged 62 g daily, and the absorption was 85 5%   In the second 12 day period, the intake was raised to 88 g daily, and the absorption was again 85 5%   Inspection of the 4-day fat excretion figures shows more variation in the two periods than the 12-day averages would imply, but the

results recall similar findings in sprue (4) and it appears that with moderate intakes the proportion of fat absorbed in steatorrhœa remains constant This may be because the stomach controls the rate at which fat reaches the intestine (6), and with added amounts of fat, though the total amount of fat reaching the intestine is increased, the rate at which it is presented for absorption remains the same

The results with 12-day balance periods were fairly consistent in the patient with tropical sprue there was a steady improvement, in the two patients with idiopathic steatorrhœa fat absorption remained the same with treatment and with changes in diet In contrast, the results of 4-day balances showed much variation and it is easy to see that results based on single 4-day periods could lead to a false conclusion of deterioration or improvement in fat absorption

TABLE IV

Fat excretion in three patients with anæmia

Fat intake 70 g per day

	4 day fat excretions g		
T R I No 66341	7 7	38 0	19 6
T (post splenectomy)	7 7	8 8	16 0
Bo R I No 38274	12 4	13 2	26 4
Br R I No 64534	32 0	34 6	30 4

The diagnosis of steatorrhœa

Prolonged balance observations are not necessary for the detection of steatorrhœa when the absorption defect is gross In the present investigation the 4-day figures for the patients with steatorrhœa were consistently above the upper limits of normal (23 g) in spite of the variability of 4-day collections, and a diagnosis could be based on the shorter collection period For the detection of slight defects in fat absorption, the longer period is necessary This is illustrated in Table IV, showing 12-day fat balance results in three patients with obscure anæmias In all of them the anæmia was corrected by transfusion before the observations were begun In patient T, a case of acquired hæmolytic anæmia, the mean 4-day fat excretion before splenectomy was 21 7 g, range 7 7-38 g The mean is significantly

different from the normal mean of 11 5 g (p ⟨ 02 by the *t* test)   Fat excretion was within normal limits after splenectomy   In patient Bo, a case of myxoedema with megaloblastic anæmia not responding completely to liver therapy, the mean 4-day fat excretion was 17 33 g (range 12 4-26 4 g)   Fat excretion was probably abnormally high (for the difference between the normal mean 4-day excretions and the mean 4-day excretion in this patient, p ⟨ 05 by the *t* test)   In the third patient (Br) who had a gastroenterostomy for duodenal ulcer, fat excretion in all three 4-day periods was abnormally high (mean 32 3 g)   In the first two patients, at least, single 4-day collections would probably not have revealed increased fat excretion   It cannot be concluded that these small increases in fæcal fat excretion necessarily signified an absorption defect ,   increased intestinal secretion and, in one patient, defective control of stomach emptying are other possible explanations   Nevertheless, the fat excretion was above normal

TABLE V

Fæcal nitrogen losses in steatorrhœa

Subject	Mean daily faecal N in three 4-day periods (g)			Mean daily faecal N (12 days) (g)	Protein intake (g)	Fat absorption %
A	2 5	1 75	2 2	2 15	70	50
With folic acid	1 7	—	1 15	1 4	70	61
With folic acid	1 7	1 0	1 55	1 4	70	77
Liver and yeast	0 9	1 9	1 5	1 4	70	85
D	1 7	2 7	2 7	2 4	70	80
H* (R I No 54888/46)	7 3	6 6	6 9	6 9	70	57
	7 3	6 5	4 2	6 0	120	45
	8 4	10 9	9 95	9 75	150	34

* The changes in fat absorption with varying protein intake in this patient were found not to be significant on analysis

Fæcal nitrogen losses in steatorrhœa

Total fæcal nitrogen excretions were determined in three patients with steatorrhœa (Table V)   In a patient with tropical sprue (A) and a patient with idiopathic steatorrhœa (D) the fæcal nitrogen loss was normal or very slightly above normal   This is the usual finding in non-pancreatic steatorrhœa, though raised values may be found with diarrhœa (32)   In patient H, the fæcal nitrogen excretion was about three times the normal

and rose to four times the normal on a protein intake of 150 g daily In this patient Dr J Newsome later demonstrated deficient pancreatic function by Lagerlof's secretin test (16), and the finding of raised faecal nitrogen excretion is in fact a diagnostic feature of pancreatic steatorrhœa (Pratt 1934) This patient's fat absorption was very deficient (Table V), but the excreted fat was almost completely split, the proportion of the total fat in the split form in the faeces ranging from 88-100% Since these estimations were done on 4-day stool collections, the high values may in part be attributed to the lipolysis that occurs in faeces on storage, particularly in faeces from patients with steatorrhœa (3), but the findings serve to emphasize the fact that the degree of splitting of the stool fats cannot be relied on to help in the diagnosis of pancreatic steatorrhœa Pancreatic steatorrhœa may be distinguished from intestinal steatorrhœa by the high faecal nitrogen loss

DISCUSSION

Very variable data on fat excretion are obtained by ordinary methods of marking and collecting stools, and fat absorption cannot be assessed with single short collection periods With several consecutive short periods the collection error is reduced and an estimate is obtained of the variability of the data In this way, fat absorption can be compared at different times in the same subjects and any differences in excretion can be assessed statistically For the diagnosis of steatorrhœa, any method that will reveal the increased fat excretion is adequate Most cases are revealed by a simple analysis of the fat content of the stool, but the proportion of fat in the stool may be normal though the total daily excretion is increased In doubtful cases the excretion of fat over a given period of time has to be measured, on a 70 g per day intake of fat, normally probably less than 23 g are excreted in a single four-day period To detect small increases in fat excretion, the excretion over several four-day periods should be measured, normally probably less than 54 g are excreted in 12 days In steatorrhœa, the total fat excretion is, within limits, proportional to fat intake, and the defective absorption of fat can be made more obvious by increasing the dietary fat (34) In normal subjects, fat excretion changes little with moderate variations in intake

Steatorrhœa is not necessarily due to a defect in intestinal absorption Cases of pancreatic steatorrhœa are distinguished by an increase in faecal nitrogen excretion Slightly increased fat excretion may result from gastric lesions (23) and possibly from an increase in intestinal secretion of fat

In a single case, our results suggest that after treatment with folic acid fat absorption in tropical sprue may return to near normal, though treatment with liver and yeast may have contributed to the improvement in the later periods of observation Treatment with folic acid for five months had no effect on fat absorption in two patients with idiopathic

steatorrhœa Davidson and others (10) reported improved fat absorption in one of two patients with tropical sprue and no improvement in other patients with steatorrhœa after treatment with folic acid Their conclusions were based on the results of single 3-day periods and the significance of their results cannot be assessed As far as we know, no adequate study of the effect of folic acid on fat absorption has appeared

SUMMARY

(1) Balance studies, in which the output of a substance is compared with the intake over a given period, must usually be carried out for a period of at least twelve days, and fats are no exception to this rule

(2) Fat excretions over 4-day collection periods have a co-efficient of variation of about 30 per cent The estimated probable upper normal limit of fat excretion in four days on a 70 g fat diet was 23 g

(3) Estimation of the 4-day excretion of fat is usually adequate for the diagnosis of steatorrhœa Where the effects of therapy are being studied, the 12-day period must be used

(4) Pancreatic steatorrhœa is distinguished from steatorrhœa of intestinal origin by the high fæcal nitrogen loss

(5) After treatment with folic acid as well as liver and yeast, fat absorption improved gradually to near normal in one patient with tropical sprue , there was no change in fat absorption after several months' folic acid treatment in two patients with idiopathic steatorrhœa

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A COMPARISON OF ESTIMATES OF CIRCULATING RED BLOOD CELL VOLUME GIVEN BY THE ASHBY MARKED RED CELL METHOD AND THE T 1824-HÆMATOCRIT METHOD IN MAN

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DYE methods of estimating blood volume have met and are still meeting criticism, and results obtained by such methods are not uncommonly viewed with some doubt   Critics suggest that (a) the dye method does not measure the true plasma volume and (b) the red cell volume as measured from the dye plasma volume and hæmatocrit is not the true red cell volume   In other work on injured men one of us has been interested particularly in (b), the error with which red cell volume is estimated by the dye method   To make a comparison between dye red cell volume and true red cell volume a means of estimating "true red cell volume" is necessary   Many authors have used the CO method for this (e g 13 and 21), but in view of the known combination of CO with substances other than the hæmoglobin of circulating red cells, it must be accounted an uncertain method of measuring the red cell volume   Alternative methods of estimating the "true red cell volume," in which the red cell marking agent is less liable to escape from the blood stream than CO, are (a) the radioactive red cell methods (12) (8) and (b) the modified Ashby method (5)   Radioactive red cell methods at this time were not available to us, but it seemed that it might be possible to use the Ashby method with considerable precision   This has proved the case, and we have therefore used this method for our estimate of true R B C volume

In what follows three groups of observations are described, two on patients and the third on normal subjects, in which the total red cell volume was measured simultaneously by the dye (T 1824) and Ashby methods In the first two groups we were concerned to get good estimates by both methods in patients having stable circulations but showing some variation in hæmoglobin and hæmatocrit levels   To secure accuracy of the R B C V

estimate by the modified Ashby method we transfused a large and easily measurable volume of " marked " red cells, approximately 540 ml of whole blood to the patients of group 1 and 540 ml of packed red cells to group 2 In the third group of observations we desired also to compare simultaneously the rates of mixing of dye and R B C in normal men Smaller volumes of blood (100-200 ml) mixed with dye were transfused rapidly These experiments are fully reported in our next paper, and only the R B C V estimates are here recorded

Before reporting our results the methods used and precautions taken are described in some detail The Ashby method has not previously been used with the precision here attained We therefore describe at some length our use of this method and the tests applied to it

Methods

(a) *The Ashby Method*

The principle of the Ashby method (1) is as follows A Group A subject is transfused with Group O red cells In his mixed blood the Group O red cells can be detected and counted after agglutinating the Group A red cells with an anti-A serum A modification of the method utilizes the hæmolysing action of anti-A serum to remove the Group A cells, for this reaction fresh complement is necessary to activate the hæmolysin If a known number of Group O red cells are transfused, and, when complete mixing in the recipient's circulation has taken place, the number of Group O red cells in unit volume of the recipient's blood is determined, the total volume of the subject's red cells may be determined from the equation

$$\text{R B C V} = \frac{\text{ND}}{\text{NR}} \times \text{H}$$

where   ND = the total number of Group O cells transfused,

         NR = the number of Group O cells in 1 ml of the recipient's Blood,

         H = the true volume occupied by the R B C in 1 ml of the recipient's mixed blood

The total number of R B,C transfused (ND) is estimated from the volume of blood transfused and the number of R B C counted in unit volume of transfused blood

Measurement of volume transfused In the first group of observations whole blood transfusions were given Shortly before the experiment a suitable donor was bled into a calibrated one pint M R C blood bottle, containing 50 ml of 2 5% tri-sodium citrate, to a few mm above a mark indicating an accurately known volume of about 540 ml

In the second group of observations packed red cell transfusions were given Blood was stored for 36 to 72 hours in disodium-citrate-glucose

anticoagulant in the cold   The plasma from 2 pints of such blood was siphoned off and the red cells were then decanted into a calibrated 1 pint M R C blood transfusion bottle, again to a few mm above the mark

The blood or concentrated red cell suspensions were mixed thoroughly and a sample was immediately withdrawn   After a second series of inversions a second sample was withdrawn   The two samples were of a volume sufficient to lower the blood meniscus in the bottle to the mark   By experiment it was found that a volume of 540 ml could be measured in this way with an accuracy of $\pm$ 2 ml , or less than $\pm$ 0 5% of the total   The hæmoglobin contents of the two samples were measured by an accurate method (19) to guard against sampling errors

To transfer into the patient this accurately known volume of blood the following method was employed

The bottom was cut off an M R C blood bottle to form a funnel   Into the neck of this bottle the rubber bung, containing glass tubes and filter of the ordinary M R C blood giving set was plugged   The delivery set was filled with saline to the level of the neck of the transfusion bottle and clipped off   The measured volume of blood in the calibrated bottle was poured into the funnel, and the last 1 or 2 ml of blood clinging to the walls of the original container were washed in with a little 0 85% saline   The needle was inserted into the patient's vein and the clip released   When at the end of the transfusion all the blood had left the funnel, the sides of the funnel were washed and the dead space of the rubber tube and dripper flushed through with a little saline to displace almost all the blood into the patient   At the end of the transfusion, at most 2 ml had failed to enter the patient's vein

The method used for the preparation, transfusion and measurement of the volume of the dye blood mixture used in the third group of experiments is described in our next paper, (3)

Measurement of red cell count of blood injected   0 2 ml from one of the samples of donor blood or concentrated red cell suspension was pipetted into a 50 ml standard flask containing 0 85% sodium chloride solution   The flask was made up to the mark with saline, thus giving a 1 in 250 dilution of the donor blood or red cell suspension   Both pipette and flask had been carefully calibrated   After thorough mixing, samples were counted by two observers (J L and D B), each using one hæmocytometer chamber standardized by the National Physical Laboratory   Each observer usually took three successive samples from the diluted blood and counted 1,000 cells in each sample   Thus, on the average, there have been made at least 6 separate counts on 6 separate samples taken from the standard dilutions of donor blood or red cells, totalling in all about 6,000 R B C , rarely less and sometimes more

Measurement of Group O red cell count in recipient   The modification of the Ashby method employed in all cases was that of Dacie and Mollison (5)

The agglutinating sera used on all but one occasion were two very potent anti-A sera, obtained from rabbits immunised with an artificial A antigen (16) The optimal dilutions for maximum agglutinating activity lay between 1/16 and 1/64 (2)   On one occasion (Subject Ba) a potent anti-B serum of human origin was used undiluted   On two occasions, to check the agglutination method, a hæmolysis method was put through simultaneously Undiluted anti-A serum, obtained from selected men immunised with purified human group A substance (14), was treated with a 1 in 10 dilution of guinea pig complement that had been suitably absorbed   This hæmolysin complement mixture was incubated at 37° C for 3 to 5 hours with the recipient's blood samples

Counts of the inagglutinable red cells (or the red cells that were not hæmolysed) were made (a) on samples of the recipient's blood drawn before the transfusion of Group O cells (to determine the " blank " count) and (b) on samples drawn after the transfusion   0 02 ml of the blood to be counted was pipetted and washed out into 2 ml of dilute serum (or serum complement mixture) delivered from an Ostwald type pipette   Both 0 02 ml and 2 ml pipettes had been calibrated and were used with much care   A 1/101 dilution of the recipient's blood was thus obtained   After absorption and centrifugation (or incubation) the mixtures were thoroughly shaken, and samples were transferred to the hæmocytometer chambers where counts were made of the numbers of free red cells   The numbers of samples and free red cells counted, and the manner of making the counts, were as described earlier for the counts on the transfused blood   The number of Group O cells in unit volume of the recipient's blood after the transfusion is taken as the inagglutinable (or unhæmolysed) count minus the appropriate blank count

Both agglutinating and hæmolysing sera were very potent   Thus, blank counts done in every experiment on samples drawn before transfusion varied between 3,000 and 55,000 per c mm   or between 0 3 and 11%, with a mean of 3%, of the total inagglutinable or unhæmolysed red cell count after the transfusions   On only 4 occasions was the blank count more than 6% of the total inagglutinable red cell count, in the experiments on subjects Bi, Ro, Ba and the last experiment *in vitro* (Table I)   To obtain such low blank counts it was necessary to select recipients by a preliminary trial of sensitivity of their red cells to these strong agglutinating sera, as well as to use very potent sera

Errors of estimation   Errors may arise in the estimation (a) of the total number of Group O red cells transfused, (b) of the total number of Group O red cells in the recipient's post-transfusion blood samples, and (c) of the hæmatocrit   As has been described great care has been taken in measuring the volume of blood transfused and in making the dilutions of transfused and recipient's bloods for counting   The method of estimating the hæmatocrit is described shortly and in any one experiment a number of

TABLE I

In vitro tests of Lehby marked cell method of estimating blood volume

A Measured Vol Mixed Blood in ml	B Marked cell blood count of added (Group O blood) $\times 10^4$ per mm³ ± CV	C Marked cell count in mixed blood $\times 10^4$ mm³ (± C V) corrected by subtraction of blank	D Blank counts $\times 10^4$ per mm³	E Vol of mixed blood calculated by method $= \dfrac{(B)}{(C)}$	F % Difference between calculated and measured $= \dfrac{E-A}{A} \times 100$	G Haematocrit of mixed blood %
5	4 007 ± 1 2%	a 0 8071 ± 1 1% b 0 8088 ± 1 1%	0 008	0 08 0 07	+ 1 3% + 1 1%	42 5
6	5 012 ± 1 3%	a 0 0175 ± 1 4% b 0 0015 ± 1 4%	0 005	5 03 5 01	— 1 2% + 0 2%	43 5
6	5 001 ± 1 3%	a 0 7001 ± 1 1% b 0 8000 ± 1 4%	0 003	0 26 0 25	+ 4 3% + 4 2%	30 7
6	4 007 ± 1 2% 4 004 ± 1 1%	a 0 8213 ± 1 3% b 0 8113 ± 1 2%	0 010	0 05 0 10	+ 0 8% + 1 7%	22 5
6	4 011 ± 1 1% 4 007 ± 1 2%	a 0 5401 ± 1 4% b 0 5407 ± 1 4%	0 010	0 20 0 18	+ 2 2% + 2 0%	42 0
6	5 080 ± 1 1%	a 0 5554 ± 1 2% b 0 5558 ± 1 2%	0 010	0 10 0 10	+ 1 8% + 1 8%	22 0
11	5 012 ± 1 3%	a 0 1003 ± 1 3% b 0 5053 ± 1 1%	0 005	10 07 10 77	— 0 3% — 2 1%	23 5
11	5 001 ± 1 3%	a 0 1550 ± 1 1% b 0 1110 ± 1 1%	0 003	10 98 11 33	— 0 2% + 3 0%	30 5
11	5 080 ± 1 1%	a 0 4570 ± 1 4% b 0 4070 ± 1 4%	0 010	11 11 10 00	+ 1 0% + 0 0%	30 5
21	4 740 ± 1 2%	a 0 2101 ± 1 5% b 0 2223 ± 1 5%	0 006	21 04 21 37	+ 3 0% + 2 0%	—
11	4 076 ± 1 3%	a 0 1134 ± 1 7% b 0 1151 ± 1 7%	0 010	13 88 13 23	+ 7 0% + 5 4%	—

Blood was mixed in the proportions of 1 ml of Group O blood to 5, 8, 10, 20 and 40 ml of Group A blood. The volume of mixed blood shown in Column A is compared with the volume calculated in Column E from the counts shown in Columns B and O. O V, the coefficient of variation $= \dfrac{\text{the standard deviation} \times 100}{\text{Mean total count}}$

Paired marked cell counts marked a and b in Column O were counts made on duplicate mixed blood samples

hæmatocrit observations have been made   The combined error from all these sources is small   Greater errors might arise either in the estimate of the numbers of red cells in the diluted samples, or in the method of estimating the number of Group O red cells in the mixed samples   To obtain a precise estimate of the number of red cells in a hæmocytometer chamber a sufficient number must be counted and these must be distributed " at random " through the counting chamber   Clearly if they are distributed in some other way a false estimate of their number may be made   To minimize error from this latter cause we have examined the distributions of the red cells in the hæmocytometer chambers by statistical methods, which for convenience are briefly summarized in an appendix to our subsequent paper (3)   These show the red cell counting technique to have been satisfactory

In estimating the number of transfused R B C in mixed blood, the recipient's own R B C are clumped together by strong agglutinating sera, the red cells are spun to the bottom of the containing tube, and the unagglutinated cells are shaken free from the clumps   Two sources of error might be expected   (a) Agglutinated red cells belonging to the recipient might be shaken free from the clumps and counted as transfused cells, (b) free transfused cells might be captured in the clumped recipient's cells, so giving too low a count of transfused cells   To minimize the first error blank counts were made as described earlier   To test the second sort of error and the volumetric accuracy of the method, we have made experiments *in vitro* in which we have tried to duplicate our experiments *in vivo*   The experiments *in vitro* were made as follows

A 20-50 ml sample of Group A blood was drawn and oxalated to represent the recipient's blood   From a portion of this the plasma was separated and was used to dilute the remainder of the sample to represent recipients with differing hæmatocrit levels   Blank counts as described were made on this Group A blood   5-10 ml of Group O blood were drawn and oxalated to represent the transfused blood and an accurate count was made on this   To an exactly measured volume of Group A blood an exactly measured volume of Group O blood was added, and the two bloods thoroughly mixed   The proportions of Group O to Group A blood ranged between 1 to 5 and 1 to 40, and are similar to the proportions used in our experiments *in vivo*   Counts of unagglutinated cells were made on the blood mixtures and corrected by subtraction of the blank counts   Knowing from the counts (a) the total number of Group O cells added to the mixture, and (b) the number of Group O cells in unit volume of the mixture, the total volume can be calculated and compared with the measured volume   In Table I are shown the results of such experiments *in vitro* with the estimated statistics   It is clear that *in vitro* the method is capable of giving precise results

In vivo Test of the Ashby Method   As a check on the reproducibility of the results obtained by the method *in vivo*, repeated estimates of total

TABLE II

Repeated estimations of total hæmoglobin in 2 subjects by the Ashby marked red cell method

Subject	No of estimation	Volume of transfused blood and red cell count per mm³ × 10⁶	Duplicate recipient's counts per mm³ × 10⁶ after transfusion	Hæmoglobin in g per 100 ml after transfusion	Total hæmoglobin in g *	Mean total Hb 2 minus mean total Hb 1	g Hb transfused for second BV estimation
RP	1	216 ml / 1 188	a 0 306 / b 0 295	a 12 7 / b 12 0	a 376 g / b 380 g / Mean 381 g	80 g	90 g
	2	510 ml / 5 707	a 1 310 / b 1 358	a 11 25 / b 11 3	a 175 g / b 158 g / Mean 167 g		
GN	1	512 ml / 3 304	a 0 342 / b 0 340	a 12 7 / b 12 05	a 683 g / b 816 g / Mean 683 g	24 g	10 g
	2	410 ml / 3 131	a 0 580 / b 0 587	a 13 2 / b 13 2	a 711 g / b 703 g / Mean 707 g		

* Calculations For subject R P total hæmoglobin in g 1 is

$$\frac{210 \times 1\,188 \times 12\,7}{0\,306 \times 100}$$

2 is

$$\frac{(210 \times 1\,188 + 510 \times 5\,707) \times 11\,25}{1\,310 \times 100}$$

hæmoglobin were made in two subjects   The estimates are shown in Table II   It will be noted that in both subjects the duplicates of each estimate lie within and in two instances well within 2% of their mean   In subject R P the sum of the first total hæmoglobin estimate and the amount of hæmoglobin given in the second transfusion is only 4 g greater than the second estimate of total hæmoglobin, or too great by less than 1% of the subject's total hæmoglobin   In subject G N the sum of the first total hæmoglobin estimate and the amount of hæmoglobin given in the second transfusion is 16 g greater than the second total hæmoglobin estimate, or greater by about 2% of the subject's total hæmoglobin   Hence the Ashby method gives precise results *in vivo*   The principle of estimating total hæmoglobin is the same as that for estimating total R B C V   The form of the calculations are shown in Table II

(b) *The Dye Method*

The accuracy of the dye method of estimating plasma volume depends on (1) an exact knowledge of the quantity of dye injected, (2) an accurate estimate of the dye content of the dyed plasma samples and, (3) the injected dye only leaving the plasma at a determined rate

We have injected an exactly known volume of dye solution thus   A calibrated very thinly oiled 20 ml syringe is filled to the mark with dye solution   After a suitable volume has been injected—usually between 12 and 20 ml —the uninjected remainder of the dye is discharged into a narrow calibrated measuring cylinder in which it is accurately measured   By this means the volume injected is readily estimated to 0 1 ml or to less than 1% of the total injected   For each plasma volume estimation an accurately prepared dilution of the injected dye was made up in the patient's undyed plasma or serum drawn just before the dye injection   This standard dilution of dye in plasma was made up to have a concentration close to that of the patient's dyed plasma samples

For estimating the dye content of plasma the method of Gibson and Evelyn (7) was used with certain modifications

Enough dye is injected to give concentrations in plasma near 1 mg %   Dye concentration was measured with a sensitive photoelectric photometer of the type described by Reeve (16) with the red colour filters used by Gibson and Evelyn (7)   Cells of 5 mm thickness have usually been used   The estimates of plasma dye concentration have ordinarily been completed by 3 hours after the withdrawal of the dyed samples from the subject

Since there is a slightly curvilinear relation between plasma dye concentration and plasma optical density, values for dye concentration of both standards and samples have been taken from carefully prepared calibration curves   There are two main causes of error in the estimation of the dye content of plasma samples, (a) the presence of free hæmoglobin pigment in plasma, (b) the presence of varying degrees of cloudiness in the plasma samples   No trouble was caused by (a) in these experiments   We have met with trouble from (b) in only a few samples indicated later, but much care has been taken in examining samples for the presence of cloudiness and in treating patients and samples so as to avoid cloudiness

The rate of loss of T 1824 from the plasma was estimated by extrapolation from the dye contents of samples drawn at intervals after the injection of dye   In the first and second groups of experiments 3 dyed samples were drawn starting at 12 18 minutes after the dye injection and with 12 18 minutes intervals between them   In the third group of experiments the dye loss is calculated from samples drawn at approximately 10, 20, and 30 minutes after the dye injection

The plasma dye concentrations of all samples have been corrected by the formula suggested by Noble and Gregersen (17)

$$Dn \times \frac{Pr^1}{Pr^n}$$

where Dn   =   the dye concentration of the *n*th sample

Pr¹   =   the total protein content of the 1st dyed sample

Prⁿ   =   the total protein content of the *n*th dyed sample

The total proteins were estimated by the $CuSO_4$ method of Phillips and others (18)   This formula corrects approximately for losses of dyed albumin, or for loss or gain of fluid by the plasma

(c) Dye Hæmatocrit

The T 1824 red cell volume estimate is derived from the plasma volume and the hæmatocrit It has been shown that a small proportion of the red cell column of the hæmatocrit consists of trapped plasma (11 22) and hence the hæmatocrit as ordinarily determined gives a small over estimate of the volume of the red cells Therefore, as shown by Root Roughton and Gregersen (21), for an accurate estimation of total red cell volume from the dye plasma volume and the spun hæmatocrit, the spun hæmatocrit must be corrected for its content of trapped plasma, or the true volume of red cells must be determined by another method In each subject, at the time of the blood volume estimations, a single estimation of the volume of red cells in unit volume of blood has been made with a *micro* T 1824 method, and this estimate has been compared with that obtained from samples of the same blood spun at 1500 × g for 30 minutes in hæmatocrit tubes Our determinations showed that the amount of plasma trapped in the spun hæmatocrit red cell column ranged from 3 to 7% Since in such determinations it is easy to make small errors in dye estimation, and such errors would account for the range of values found, the spun hæmatocrits of all blood samples have been corrected to allow for a *mean* value of 5% of trapped plasma, i e by multiplying the hæmatocrit by 0 95

In a part of the first undyed and heparinised sample of the blood the volume of red cells in unit volume of blood was estimated with T 1824 The following micro method was employed.

The undyed blood was divided into two portions Plasma was separated from one part, and to 1 5 ml of this plasma 0 02 mL of 0 2% T 1824 made up in physiological saline was added from a Burroughs Wellcome " Agla " microsyringe fitted with a curved 26 S W G needle Dye and plasma were mixed and acted as a dye standard Exactly 5 mL of blood from the other portion was placed in a 5 ml measuring cylinder, and 0 04 ml of the same dye solution was added from the same microsyringe Dye and blood were mixed by careful inversion for 10 minutes and the resulting dyed plasma was separated by a short period of centrifuging at 1500 r p.m in a small angle centrifuge The dye contents of standard and sample were then determined as precisely as possible by the photoelectric method used in the plasma volume estimates From the concentrations of dye in the standard and in the plasma from the dyed blood the volume of plasma in 5 ml of blood is calculated In such a micro method a precise knowledge of the volume of blood, plasma and added dye solution is required The volume of blood, 5 ml was measured precisely to a mark and also checked by weighing and dividing the weight by the determined specific gravity The volume of plasma used for the dyed plasma standard was determined by weighing the plasma and dividing by the determined specific gravity of the plasma Specific gravities of blood and plasma were determined carefully by the method of Phillips and others (18) The microsyringe was used with much care It was held horizontally in a stand, its needle was curved and the junction between the needle and syringe was lightly vaselined Dye was delivered at a constant very slow rate of 0·01 ml per minute Drops were delivered just above the plasma or blood and gently wiped off on the side of the glass container The glass surface has a much greater attraction for the drop of 0 2% dye than the polished flush-cut tip of the needle, and dye can be completely transferred from needle tip to glass container As a check a series of 0 04 mL samples of 0 2% T 1824 were delivered from the microsyringe into equal quantities of bicarbonate solution, and after mixing the dye concentrations were determined with a sensitive photoelectric photometer No significant difference in the dye concentrations was detected.

In normal subjects Gregersen and Schiro (11) found a mean value of 4 2% of trapped plasma and Stohl and Hunter (22) a mean value of 4 5% Chapin and Ross (4) found a considerably higher value averaging 8 to 9% of the red cell column Working in our laboratory Miss B Morrison making triplicate estimations on the blood of normal subjects with the micro T 1824 method just described finds values varying from 3 to 5% with a mean of about 4% thus confirming the work of Gregersen and Schiro (11) Since from our experience, we think that the value of blood (such estimates occasionally vary from 2 5 to 6 0%) is slight differences in plasma opacity, and since the manipulations involved in the method tend to increase slightly the opacity of the plasma separated from the dyed blood as compared with the dye standard, and this tends to give too low values we prefer the value of 5% trapped plasma A possible explanation for the results of Chapin and Ross (4) is presented in our subsequent paper (3)

(d) General technique, other than already described

The transfused blood and the recipient's blood were cross matched and Rh typed Blood samples were drawn without stasis into very thinly oiled syringes In the first two groups of experiments the samples were divided, the major part was allowed to clot to provide serum samples for dye and protein estimations a small portion was heparinized for hæmoglobin and hæmatocrit estimations In the third group of experiments the whole blood samples were heparinized and all estimations were made on heparinized blood or plasma Heparinized plasma samples are satisfactory for dye estimation provided that the concentration of heparin is the same in all the plasma samples of a plasma volume estimate The hæmoglobin concentration and

hæmatocrit of all blood samples were estimated, the first as described in (19), the second by spinning at 3,000 r p.m for 30 minutes in a centrifuge of radius 15 cm (approximately 1500 × g) in hæmatocrit tubes of 3 mm diameter and 10 cm length   In the first group of experiments hæmoglobin and hæmatocrits were estimated in duplicate   In the second group of experiments single estimations only were made on each sample   On each plasma and serum sample the specific gravity was measured by the CuSO$_4$ method (18)

Description of the experiments

To avoid plasma cloudiness all experimental subjects were starved for at least 12 hours before the experiment   In the first two groups of observations to avoid disturbing the circulation by transfusion reactions either constrictive or urticarial, and hence possibly disturbing the plasma volume estimate, we transfused rather slowly over the course of 45 to 90 minutes, the patients were kept well warmed, and about half of them were given injections of ¼ gr (16 mg) morphine, a half to 1 hour before transfusion started   It has been shown that morphine does not affect plasma volume estimations with the dye T 1824 (20)   Before the beginning of transfusion a sample of the patient's blood was taken for an estimate of the blank count Whole blood or packed cells were than transfused as described and the last portions washed in with saline   Half to one hour after the end of transfusion, when it had been ascertained that there was no reaction, a plasma volume estimate was made with T 1824 as described   Counts of transfused Group O cells were made on the first and last dyed samples, there being approximately a 30 minute interval between the drawing of these two samples   Before and during the experiments all patients were recumbent in bed   The experimental procedure used in the third group of observations is described fully in our next paper (3)   In these experiments there was a period of 10 to 30 minutes for mixing of transfused and patient's red cells after a transfusion lasting for 1 to 2 minutes, compared with a period of 30 to 90 minutes after a transfusion lasting an hour in the first two groups of experiments   In the third group of experiments no subject showed an urticarial reaction

Results of experiments

Table III A summarizes the results of 8 experiments in which whole blood was transfused   Duplicates given by the hæmolysin method are shown for two samples in two cases   The first 6 of these experiments were technically good, but in the last 2, patients E and Wt, a small degree of cloudiness developing unequally in the dyed plasma samples, throws in doubt the precision of the dye estimate   It is thought, however, that these two dye estimates are not much in error   It will be seen that on the average the dye method measures a 12% higher volume of red cells than the Ashby method   Brief clinical notes on all patients are shown in Table IV   The patient N experienced a mild urticarial reaction during the estimation

In transfusing 540 ml of Group O blood to Group A patients 250 to 300 ml of plasma containing anti-A substance were transfused   To minimize possible effects due to the transfusion of this foreign plasma, in the second group of cases 540 ml of packed red cells were transfused   The results are shown in Table III B   The fourth patient (Mc) suffered from an inflammatory mass in the abdomen and showed an increased rate of loss of dye from the plasma, but probably the dye estimate of his plasma volume is not grossly in error   Here on the average the dye method gives a 17% higher red cell volume estimate than the marked cell method

In Table III C are shown the results of estimates by both methods on the third group of 8 normal subjects   Since smaller volumes of blood mixed with dye were rapidly transfused, we think that these experiments were more liable to small errors in estimates of the total volume of marked red cells and dye transfused   The results show a rather greater scatter in the difference between estimates given by the two methods, but the average of a 12% greater estimate by the dye method agrees with the results in the patients of Table III A

Discussion

We, as others (9, 12) find therefore that the T 1824 method gives an estimate of total red cell volume averaging 12 to 15% higher than the " true " red cell volume   Why is this ?   First the estimate of the true red cell volume might be too low   It has been shown that the Ashby method as here used gives results of considerable precision *in vitro*, and therefore, if it gives too low results, these must be due to causes *in vivo*   The volume of injected marked red cells is measured accurately and therefore too low results must be due to an apparent gain of marked red cells *in vivo*   There are only two possible causes of this—incomplete mixing of marked red cells in the circulation or gross technical errors   Since in the first group of experiments the dye determination was made some interval after the marked red cell transfusion had been completed, and in the second group of experiments both dye and red cell estimates of red cell volume were made simultaneously, incomplete mixing cannot be used as an explanation, and the comparable results of others using radioactive methods make technical errors unlikely (9, 12)

The reason for the discrepancy must therefore be sought in the dye method   There are two possible causes, (a) a proportion of the injected dye might be lost from the circulation, (b) there might be varying proportions of red cells to plasma in the blood vessels of the body   Opponents of the dye method usually favour explanation (a), and users explanation (b)

Concerning (a), in section L of Tables A, B and C are shown the percentages of injected dye that must be lost in each experiment, if the sole cause of the T 1824 overestimate of red cell volume is uncorrected dye loss

TABLE IIIa

Comparison of total volume of red cells measured by T 1824 and by Ashby method
Patients transfused whole blood

Patient	Dye haematocrit %	T 1824		RBCV by marked cell method Calculated from samples 1 and 3		RBCV dye × 100 / RBCV marked cells for samples 1 and 3		Dye BV × 100 / Total BV	L	M	% of initial BV transfused
		Plasma V ml	RBCV ml	S 1 ml	S 3 ml	S 1	S 3				
S	42.6	3080	2280	2000	2040	112	112	105	12	7	10%
Gu	39.0	1960	1250	2070 / 1150 / 1130*	1160 / 1150*	109* / 111* / 121	108 / 109* / 121	103	8.5	5	18%
N°	38.7	4040	2540	2090	2100	108	109	107	18.5	12	8%
A	35.6	3590	1980	1840	1810	106	107	103	8.5	5.5	10%
Be	35.1	4010	2170	2040	2020	105*	103*	103	6.5	4.5	9%
Ws	34.8	2460	1310	1250 / 1200*	1270 / 1170*	109*	112*	102	7	4.5	10%
E§	41.2	3100†	2160	1800	1840	120	117	107	16.0	10	10%
Wt§	35.2	3210†	1740	1460	1460	119	110	106	16.0	11	11%

TABLE IIIb

Comparison of total volume of red cells measured by T 1824 and by Ashby method
4 patients transfused packed red cells

Patient	Dye haematocrit %	T 1824		RBCV by marked cell method Calculated from samples 1 and 3		RBCV dye × 100 / RBCV marked cells for samples 1 and 3		Dye BV × 100 / Total BV	L	M	% of initial BV transfused
		Plasma V ml	RBCV ml	S 1 ml	S 3 ml	S 1	S 3				
J	42.5	2770	2040	1780	1690	114	120	107	16.0	10	12%
C	40.2	2660	1790	1540	1530	116	117	106	14.0	9	13%
Ba	33.0	2070	1460	1250	1290	117	113	105	13.0	9	13%
Me§	35.7	2950	1630	1310	1350	124	121	107	13.0	13	13%

TABLE IIIc

Comparison of total volume of red cells measured by T 1824 and by Ashby method

Normals transfused whole blood

Volumes of 3 to 5% of the subjects' blood volumes were transfused

| Subject | Dye haematocrit % | T 1824 | | R B C V by marked cell method calculated from samples 1, 2 and 3 | | | $\frac{R B C V \text{ dye} \times 100}{R B C V \text{ marked cells}}$ | | | $\frac{\text{Dye B V} \times 100}{\text{Total B V}}$ | L | M |
		Plasma V	R B C V	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3			
Vi	40 0	2580	1720	1470 (8)‡	1400 (12)‡	1400 (30)‡	117	115	118	106	14 5	9
Gr	10 5	3380	2230	1070 (8)	1960 (11)	1910 (30)	113	114	117	105	13	8
Ro	40 2	3800	2550	2300 (8)	2550 (12)	2520 (30)	108	100	101	101	3	2
Bo	37 7	2860	1720	—	1380 (15)	1430 (30)	—	125	120	107	18 5	12
Br	44	3400	2070	2280 (7)	2290 (14)	2300 (30)	117	117	113	108	13 5	9
Ba	41 5	3320	2300	2040 (8)	2030 (12)	2050 (30)	116	116	115	108	13	8
Ro	44 2	3170	2520	2420 (8)	2300 (12)	2460 (30)	104	105	102	102	3 5	2
Ra	43 5	2780	2140	1080 (8)	2130 (12)	2040 (30)	108	101	105	102	4 5	2 5

Total B V = Dye plasma volume + mean marked red cell R B C V

$$L = \frac{\text{Dye plasma volume} - R \times \text{dye plasma volume}}{\text{Dye plasma volume}} \times 100 \; (\textit{see text})$$

$$M = \frac{\text{Dye plasma volume} - R \times \text{dye plasma volume}}{\text{Total B V}} \times 100 \; (\textit{see text})$$

$$R = \frac{\text{Marked red cell R B C V}}{\text{Dye hematocrit R B C V}}$$

(shown in the above 2 equations)

* These results were obtained with the haemolysin method

° This patient developed a mild urticaria during and after the transfusion of marked red cells

§ Less reliable results, see text

† A moderate but unequal degree of cloudiness developed in the plasma samples

‡ Figures in brackets marked ‡, and similar bracketed figures in columns beneath, indicate the number of minutes after transfusion of the dye and blood mixture at which the sample was drawn

The average for all experiments is 12% of the total dye injected   In our subsequent paper (3) certain aspects of the dye method are examined further   The experiments there reported do not exclude dye loss, but suggest, in the absence of direct proof of appreciable dye loss, that only very small amounts are lost from the plasma and go uncorrected in the method

Concerning (b), there is direct evidence that blood in the small vessels has a lower hæmatocrit than blood in the large vessels   Thoma, Krogh (23, 15) and others (e g   24) have observed that in blood flowing through the minute vessels there is a marginal plasma layer, with thickness about the diameter of a red cell, separating the vessel wall from an axial stream of blood   Fahraeus (6) has shown that when blood of constant composition is passed through fine glass capillaries of varying diameters of the order of 500 μ or less, on sealing the capillaries and determining their hæmatocrit by centrifugation, as the capillaries narrow so the proportions of red cells to plasma decrease   Hence there is both direct observation and experiment *in vitro* to support the view that there is a greater proportion of plasma to red cells in the minute vessels than in the larger vessels   The circulating blood may therefore for convenience be divided into two parts, the major part consisting of blood with the large vessel hæmatocrit, a small part consisting of plasma only which may be termed " marginal " plasma   The question arises, what proportion of the total volume of the circulation does this " marginal " plasma occupy ? This question cannot at present be answered with certainty, but maximum values can be calculated

If it is assumed that the dye method gives close to the true estimate of plasma volume, and the Ashby method as here used gives close to the true estimate of red cell volume, then the volume occupied by the " marginal " plasma may be calculated from these experiments   The results of such calculations are shown for each experiment under column M in Tables III A, B and C, expressed as percentages of the total blood volumes   It can be seen that on this assumption the " marginal " plasma occupies from 2 to 13% of the total blood volumes, with a mean of about 8%   It is shown in Table V, which is discussed shortly, that when similar calculations are made from the results of simultaneous estimations by T 1824 and other marked red cell methods, excepting only the carbon monoxide marked red cell method, on the average about the same percentage of the total blood volume is occupied by " marginal " plasma   It seems probable that the Ashby and radioactive red cell methods give results close to the true total red cell volume   Since, however, the dye method may give a small overestimate of plasma volume these values for marginal plasma can only be regarded as maximum values

Various workers have attempted by other methods to measure the proportion of red cells to plasma in the minute vessels, and the volume of

blood in the minute vessels, but in our view with no great success   The latest attempt by Gibson *et al* (10) deserves brief note

Using different radioactive isotopes to label red cells and plasma protein, these workers have attempted to estimate the total amounts of red cells and plasma in the small vessels of a variety of tissue of dogs   After preparation with isotopes the dogs were killed, and their organs removed and weighed   Then very small pieces of tissue, cut to avoid the larger vessels, were removed and analysed for their red cell and plasma content   These small pieces of tissue bled and were allowed to bleed   There are two serious criticisms of this work   First it is known that on death blood is redistributed, passing from the high pressure arterial side of the circulation to the lower pressure venous side   Secondly the chance of blood loss from vessels of all sizes in such small pieces of tissues must be very great, and from small vessels the loss would naturally be of the axial blood , any loss of small vessel blood would thus exaggerate the plasma content at the expense of the red cell content   The great variation from animal to animal both in the blood content and the authors estimate of the ' small vessel " hæmatocrit of the tissues, strengthens these criticisms

TABLE IV

Brief notes on patients on whom R B C V estimations were made

Patient		Age	Clinical Notes	Hb g %	% predicted normal B V *after* transfusion
S	♂	27	Recovering from appendicectomy	15 1	N*
Gu	♀	58	„ , strangulated hernia	13 2	N
N	♂	31	, sprue	12 7	N
A	♀	54	, bleeding fibroids	9 9	N
Be	♂	40	, „ hæmatemesis	10 7	N
Ws	♂	37	Chronic sepsis	11 2	80—90%
Γ	♂	60	Under treatment for gastric ulcer	13 1	N
Wt	♂	46	Convalescent from partial gastrectomy	11 7	N
J	♀	50	Recovering from bleeding fibroids	13 5	N
C	♂	63	, hæmatemesis	13 0	80—90%
Ba	♀	36	„ , bronchitis	10 I	N
Mc	♂	40	Inflammatory mass in abdomen	11 5	80—90%

*N — within ± 10% of predicted normal.

It is of interest to compare the results reported here with those of three other groups of workers

Gibson and others (9) have made simultaneous estimates of plasma volume with T 1824, hæmatocrit and marked red cell R B C V, using radioactive iron as the red cell marker, in 40 normal subjects   Hevesy and others (12) have made simultaneous estimates of T 1824 plasma volume,

hæmatocrit and marked red cell R B C V using radioactive phosphorus as the red cell marker in *8* normal subjects

In neither series of experiments were the hæmatocrit values corrected for the plasma trapped in the red cell column, which results in small errors in the estimates of total red cells by dye and marked cell methods, and of total blood volume The results of Gibson and others (9) have therefore been recalculated, assuming that 5% of their hæmatocrit red cell column consists of plasma We are informed by Dr Koster that there is a misprint in the paper of Hevesy and others (12), the results given in Table VI under the column headed " Dye method " being volumes of red corpuscles in ml, and not, as stated, grams of corpuscles The original dye estimates of plasma volume are not given, and Dr Koster has informed us that they were lost during the German occupation of Denmark The plasma volumes are therefore calculated from the difference between the blood volumes estimated by the dye method given in Table VII, and the corpuscle volumes estimated by the dye method taken from Table VI of the paper Hevesy and others (12) state that the centrifugal force and time of spinning used resulted in their hæmatocrit red cell columns containing 3% of trapped plasma Therefore both dye and P_{32} estimates of R B C V have been corrected to exclude this

Root and others (21) report 14 simultaneous estimates of plasma volume with T 1824, hæmatocrit and marked red cell volume using carbon monoxide as the red cell marker in 8 subjects Their results were corrected for plasma trapped in the hæmatocrit red cell column estimated as 4% of the total column

The results of these workers and our own are summarised in Table V The following mean percentages or ratios for each group of results are shown, and where they may reasonably be calculated, the standard deviations about the mean

$$(1) \quad \left\{ \frac{\text{R B C V estimated by T 1824}}{\text{R B C V estimated by marked red cells}} \times 100 \right\} - 100$$

$$(2) \quad \left\{ \frac{\text{Blood volume estimated by T 1824}}{\text{Total Blood Volume}} \times 100 \right\} - 100$$

where total blood volume is the sum of the dye plasma volume and the marked red cell R B C V

$$(3) \quad \frac{\text{R B C V estimated by marked red cell}}{\text{R B C V estimated by T 1824}}$$

This ratio is termed R and is shown under Column R

(4) The mean estimate of maximum dye loss as earlier defined This is calculated from

$$L = \frac{\text{Dye plasma volume} - R \times \text{dye plasma volume}}{\text{Dye plasma volume}} \times 100$$

and is shown in Column L

TABLE V

Comparison of certain mean values obtained from simultaneous estimates of dye plasma volume haematocrit and marked red cell RBC1 made by 4 groups of workers

Authors and numbers of observations Marked red cell method	% RBCV overestimate by dye method (1)	% BV overestimate by dye method (2)	$R = \dfrac{RBCV \text{ marked red cell}}{RBCV \text{ dye hematocrit}}$ (3)	L = estimated maximum % dye loss (4)	M = % of total BV occupied by "marginal" plasma (5)
Gibson et al (9) 10 normals Radioactive iron	+15% s d ±8	+5% s d ±2.5%	0.87 ± 0.055	13% ± 9	8% ± 4
Hevesy et al (12) 8 normals Radioactive phosphorus	+10%	+6%	~0.80	14%	0%
Root et al (21) 14 observations 8 normals Carboxyhemoglobin	+1%	+1%	About 0.99	1–2%	About 1–2%
Barnes et al (This paper) 8 normals 12 patients Ashby	+13%	+5%	0.88, s d ±0.06 (normals 0.80)	12%	8%

For definitions, see text

(5) The mean estimate of the percentage of the total blood volume occupied by " maiginal " plasma as earlier defined This is calculated from

$$M = \frac{\text{Dye plasma volume} - R \times \text{dye plasma volume}}{\text{Total blood volume}} \times 100$$

and is shown under Column M

R in the equations shown in (4) and (5) is the value calculated in (3)

Examination of Table V shows that whereas the values for the ratio R given by the Ashby method and the two radioactive methods are in close agreement, and are about 0 87, the CO method gives a mean value for R close to 1 0   Hence, assuming that each group of workers used the dye method in a comparable manner, it seems that the carbon monoxide method gives an overestimate of red cell volume of about the same order as the dye method   In view of the known ease of escape of CO from the circulation, and the presence in the body of substances other than red cell hæmoglobin that have a strong affinity for CO, and despite the extrapolation method used to correct for CO loss in the experiments of Root and others (21), it is much easier to explain the results shown as due to loss of CO from the blood, than to explain the other 3 series of results as due to an underestimate of red cell volume

Excluding then the results obtained by the CO method, it may be concluded that in normal subjects as shown in Column (1) on the average the dye method gives about a 15% overestimate of total red cell volume and as shown in Column (2) about a 5% overestimate of total blood volume, where this is taken as the sum of dye plasma volume and marked red cell R B C V   Since, on the whole, the dye method is the most generally applicable and the easiest in use of the present methods for estimating plasma and red cell volume, the values shown for R under Column (3) are the most useful of those shown in the table   The standard deviation for R is close to 0 06   From R the approximate " true " red cell volume can be determined thus

$$\text{" True " R B C V} = R \times \text{dye R B C V}$$

Total blood volume can also be determined as plasma volume + R × dye R B C V   Further the two values L and M of theoretical interest discussed earlier can be derived as shown above

It is of interest that in the Ashby and radioactive red cell methods, which on the whole show good agreement, varying quantities of marked red cells were transfused   About 20 ml of blood containing marked red cells were injected by Hevesy and others (12), about 100 ml by Gibson and others (9) and amounts varying from 100 to 550 ml by ourselves   These differences in amount transfused appeared to result in no great differences in distribution of red cells and plasma   It will be noted that our results include both normals

and hospital patients   The ratios for the 8 normals are shown bracketed in Table V, and it can be seen that there is no great difference between these and the group dealt with together   It does not follow, however, that normal subjects and patients suffering from various diseases will show the same average values for the ratios given in Table V   Before this can be stated much further work will require to be done

SUMMARY

(1)   A method for making precise estimates of red cell volume with the Ashby technique is described in detail

(2)   Possible errors of this method are examined   Both *in vitro* and *in vivo* the method gives precise results

(3)   With a micro dye method for estimating the volume occupied by the cells in unit volume of blood it is found that on the average 5% of the red cell column of the hæmatocrit spun at 1500 × g for 30 minutes consists of plasma

(4)   In 12 patients and 8 normal subjects simultaneous estimations were made of the T 1824 plasma volume, the spun hæmatocrit, the T 1824 hæmatocrit and the Ashby red cell volume

(5)   On the average the red cell volume calculated from the plasma volume and hæmatocrit corrected for trapped plasma was 13% greater than the Ashby estimate

(6)   The reasons for the greater estimate by the dye method are reviewed and it is concluded that it is due to an overestimate by the dye method and not to an underestimate by the Ashby method

(7)   Calculations are given (a) of the average amount of dye loss required if the discrepancy is entirely due to dye loss, (b) of the maximum percentage of the total blood volume occupied by the " marginal" plasma if the discrepancy is entirely due to unequal distribution of red cells in small and large vessels

(8)   Comparison of the results given by the dye method and by the marked red cell method in the series here reported and in 3 series reported by others shows that the dye method gives on the average about a 15% greater estimate of the red cell volume than the Ashby and two radioactive methods but about the same estimate as the CO method   It is concluded that the CO method gives an overestimate of red cell volume

(9)   Ratios from which the total blood volume and the approximate " true red cell volume " may be calculated from the dye plasma volume and the corrected hæmatocrit are given, and also other ratios of theoretical interest

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OBSERVATIONS ON THE ESTIMATE OF THE CIRCULATING RED BLOOD CELL VOLUME IN MAN GIVEN BY T 1824 AND THE HÆMATOCRIT, WITH SPECIAL REFERENCE TO UNCORRECTED DYE LOSS FROM THE CIRCULATION

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In the previous paper (1) it was shown that in normal subjects the dye method always overestimates red cell volume as compared with the Ashby and radioactive red cell methods, but gives an estimate of about the same size as the carbon monoxide method It was concluded that the carbon monoxide method overestimates red cell volume, and argued that the discrepancy between the T 1824 estimate of red cell volume and the Ashby and radioactive red cell methods was due to either uncorrected loss of dye from the circulation, or unequal distribution of red cells and plasma in the circulating blood It seemed to us that the latter explanation has been too readily accepted as the cause of the whole discrepancy (though there is strong evidence to suggest that it is the cause of at least some of the discrepancy), and that an insufficient examination has been made of possible dye loss In this paper we report experiments in which we have attempted to find evidence of dye loss immediately following dye injection (Section II) and during the " mixing phase " of the dye dilution curve (Section I) We also have examined the extrapolation method of correcting for dye loss (Section III), and the effects of undetected coloured impurities in the dye solutions injected (Section IV) One of us (E B R) is chiefly responsible for the observations and argument advanced in Sections II, III and IV

I —POSSIBLE DYE LOSS DURING THE " MIXING PHASE "

In Fig 1 Curve D is shown a typical plasma dye dilution curve in which concentrations of the dye T 1824 are plotted against time It can be seen that there is an early rapid fall in concentration, part A of the curve,

followed by a slow regular fall of dye concentration, part B of the curve
Since the work of Gregersen, Gibson and their collaborators (12, 9) it has
been customary to assume that during part A of the curve the dye is mixing
with the circulation, and that during part B of the curve the dye is completely
mixed but is being slowly removed   The slope of part B of the curve is
taken as a measure of the rate of removal of dye from the circulation   It
is assumed that dye is lost from the circulation at the same rate during
part A of the curve, and therefore the concentration of dye if it had been
instantaneously mixed at zero time is calculated by extrapolation

If these assumptions are correct, then part A of the curve represents
the rate of mixing of dye in plasma superimposed on the rate of dye loss
from plasma   It has, however, been suggested that the shape of part A
of the curve is not due to mixing but to earlier more rapid removal of dye
from plasma (5), though criticisms of this work have been advanced (20)
Further evidence which at first sight might support this view may be
derived from experiments such as those of Nylin (15, 16) on the rate of
mixing of tagged red cells in the circulation, which suggest that mixing is
nearly complete after one or two minutes   If part A of the curve is indeed
due to an increased rate of dye loss from the circulation this might account
at least in part for the overestimate of red cell volume by the dye method
In the experiments now to be reported we have therefore compared the
rates of mixing of dye and marked red cells simultaneously injected into
the circulation

Description of experiment

5 ml of physiological saline containing 30 mg of T 1824 were mixed with 200 to 250 ml
of freshly drawn citrated Group O blood   The total volume was measured in a sterile measuring
cylinder, a sample was taken for the estimation of the number of Group O cells transfused, and
the remainder transferred to the improvised funnel described in our previous paper (1)   A
fit, normal Group A recipient, who had fasted for 12 hours, was first thoroughly warmed in a
well heated room to the point of sweating, so that there was a considerable vasodilatation in
the vessels of his forearms   He was now placed on a couch, thickly covered with blankets,
and kept warm   We found this necessary (a) so that we could transfuse blood rapidly, (b) to
minimize any vasoconstrictive influences of the transfused blood   A wide bore transfusion
needle was introduced into a large antecubital vein of one arm, and after a preliminary control
sample had been withdrawn, was connected with the funnel containing blood mixed with dye
A wide bore sampling needle, fitted to a small syringe containing a small amount of a strong
sterile solution of heparin, was introduced into a large antecubital vein of the other arm   By
sucking a few ml of blood into the syringe and gently flushing the sampling needle with small
quantities of blood containing heparin the needle could be kept patent for 30 minutes   When
samples were required a 10 ml syringe was substituted for the small syringe   At zero time
the transfusion was started   From 100 to 200 ml of dyed blood were transfused in the course
of 1 to 2 minutes   The volume of blood mixed with dye was measured by measuring the
volume left in the funnel and transfusion tubing at the end of the transfusion in a measuring
cylinder, and subtracting this from the original volume   Samples were withdrawn without
stasis beginning at about the following times after the end of transfusion—30 seconds, 1, 3, 5,
8, 12, 20, 30 minutes   Approximately 10 ml samples were withdrawn, usually as nearly as
possible at a uniform rate over a period of 10 to 15 seconds   The samples were mixed in the
withdrawal syringe, 2 ml were transferred to oxalate tubes for counts of transfused Group O
cells, the rest was heparinized and the haemoglobin content, haematocrit, plasma protein and
T 1824 content estimated as described in our previous paper (1)   Between the time of adding
the dyed blood to the funnel and the transfusion of this blood there was an interval of not more
than 5 minutes, so that only a small amount of sedimentation in the transfused blood could
occur

Two subjects who had been insufficiently warmed before the transfusion showed vasoconstriction immediately following the transfusion In both withdrawal of the first few samples was thus made difficult and they have been excluded from this paper In the other six subjects who had been thoroughly warmed there was no difficulty in withdrawing samples No other reactions to the transfusion were noted either during or for several hours following transfusion. One subject, Ba, was bled of 800 ml before being transfused with the blood and dye mixture

Results

In presenting the results the concentrations of marked R B C have been corrected by multiplying the R B C values observed by $\dfrac{\text{Hb of initial sample}}{\text{Hb of sample in question}}$, and the plasma T 1824 values by multiplying by $\dfrac{\text{Plasma protein content of the initial sample}}{\text{Plasma protein content of sample in question}}$

Over a short period of time the circulating blood may show fluctuations either in its total volume or in the total quantities of its constituents Such fluctuations may alter the concentrations of either marked red cells or T 1824 or both Since we are interested in the relative rates of dilution of marked R.B C and T 1824 it is necessary as far as possible to correct for such fluctuations

Consider first the marked red cells Since they only differ from the recipient's red cells by a group difference and are so far as is known in every other respect identical, these are evenly and rapidly distributed throughout the whole mass of the recipient's cells Besides the degree of mixing with the recipient s red cells, changes in marked cell concentration can also be caused by addition to or removal of fluid from the circulation, or by removal of red cells from or addition of fresh red cells to the circulation. To correct for the influence of such fluctuations it is necessary to know the rate at which marked red cells would have mixed with the recipient s red cells had those red cells remained constant in quantity and suspended in constant volume of fluid Since the change of concentration of marked red cells during mixing is not great and they form only a small proportion of the total red cells, an observed number of marked red cells in a particular sample may be corrected by the following formula

$$\frac{\text{Marked R B C count}}{\text{of sample}} \times \frac{\text{Hb of initial sample}}{\text{Hb of sample in question}}$$

It is clear that changes in hæmoglobin concentration will exactly parallel changes in marked R B C count caused by addition or removal of fluid from whole blood and hence by this method we can correct to constant volume If for any reason red cells are removed from the circulation, marked and recipient s red cells so far as is known will be equally removed, and thus we can also correct for such removal to constant total quantity of red cells The equation however gives a wrong correction if recipient's red cells are added to the circulation during the course of the experiment There is no evidence that red cell stores of significant size exist in normal man (6 16 17) Correcting to hæmoglobin rather than to hæmatocrit values is preferred, since the latter may be independently influenced by changes in the volume of the red cells

Rawson (19) has shown that T 1824 is very strongly attached to plasma albumin in the concentrations used here The mixing of T 1824 in plasma may therefore be regarded as the distribution of T 1824 tagged albumin molecules amongst undyed albumin molecules Applying similar arguments to those used for R.B C, fluctuations in T 1824 concentration due to loss or gain of plasma fluid, or loss of albumin can be corrected by the following formula

$$\frac{\text{T 1824 concentration}}{\text{of sample}} \times \frac{\text{Initial sample albumin concentration}}{\text{Albumin concentration of sample in question}}$$

Noble and Gregersen (14) have proposed a fairly satisfactory compromise which avoids the difficulty of accurate albumin estimations thus

$$\frac{\text{T 1824 concentration}}{\text{of sample}} \times \frac{\text{Initial sample total protein}}{\text{Total protein of sample in question}}$$

For estimating total protein the copper sulphate method of Phillips et al (18) was used The latter formula corrects satisfactorily for loss or gain of fluid by the plasma, less satisfactorily for loss of albumin unassociated with loss of globulin, and gives a false correction for addition of fresh undyed albumin to the circulation

All subjects but Re showed very similar changes in the hæmoglobin and plasma protein contents of their blood samples. Both hæmoglobin and plasma total protein contents fell usually in very close parallel, to levels that were in the last samples drawn about 96% of the values in the first dyed samples. Subject Re alone showed very little change, not greater than a 1 5% fall in concentration of hæmoglobin and plasma protein in his series of blood samples

In Fig 1 are shown the results of a typical experiment on Subject Ra 192 ml of Group O blood mixed with dye were transfused in 70 seconds Samples were withdrawn over a period of 35 minutes at the intervals shown in the figure, in which the approximate period taken for withdrawal of each sample is also shown. Dye is lost slowly from the circulation and according to Gregersen and Rawson (12) the best measure of this loss in the first hour is the slope of the line drawn through the logarithms of the concentrations
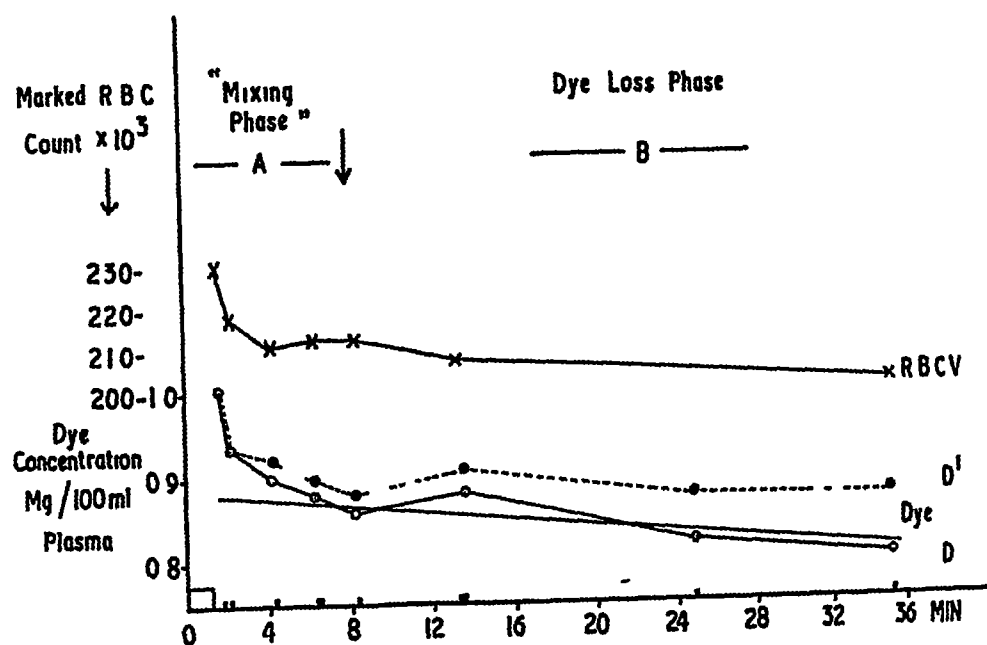
Fig 1 Simultaneous marked red cell and T 1824 dilution curves Subject Ra At zero time dye and blood containing marked red cells were transfused The concentrations of marked red cells and dye are plotted against time of sample withdrawal The method of correcting for dye loss is illustrated (*see* text)

☐ Duration of transfusion of dye blood mixture
✕ Marked red cell counts
◯ T 1824 concentrations
● T 1824 concentrations corrected for dye loss
■ Time of sample withdrawal
    Width of mark shows length of time over which sample was taken

of dye plotted against time Provided there is no great change in dye concentration there is however very little difference in the slope of the line obtained by this method and that obtained by extrapolating through the observed dye concentrations plotted against time, and the latter is simpler To enable a comparison to be made between the dilution curve of marked

R B C and of dye, we have corrected curve D for dye loss in the following manner We have drawn the best straight line we could through the last 4 dye concentrations of curve D and assumed that the slope of this is a fair measure of the rate of dye loss The observed dye concentrations of curve D have each been corrected by adding to them the dye loss estimated from the slope, thus obtaining the *dye dilution curve* D 1

Further to facilitate comparison between dye and R B C dilution curves such as those shown in Fig 1, we have reduced them to a common scale This transformation is shown in Fig 2 The mean value of the last 3 values of the marked R B C counts we have called 100 and expressed the earlier values as percentages of this, thus

$$\frac{\text{Observed marked R B C count}}{\text{Mean of last 3 marked R B C counts}} \times 100$$

The mean dye concentration of the last 4 dyed plasma samples corrected for dye loss we have also called 100 and corrected all other concentrations of dye *corrected for dye loss* thus

$$\frac{\text{Observed dye concentration corrected for loss} \times 100}{\text{Mean of last 4 dye concentrations corrected for dye loss}}$$

In Fig 2 are shown six of such pairs of curves In 4 of the experiments, those on subjects Vi, Re, Ra and Ba, it is clear that there is close correspondence between the R B C and dye dilution curves In no sample does the difference between the dye and marked red cell estimate of the course of dilution exceed 4% It should be remembered that the errors of the method include not only errors of estimation of marked red cell and dye content, but also of dye loss by extrapolation, and of hæmoglobin and plasma protein estimation If the standard error of a single value obtained by either method is taken as $\pm$ 3% (probably too low a figure), then the standard error of the difference between a pair of observations given by the two methods will be rather more than $\pm$ 4% In these four experiments, therefore, it is probable that dye and red cells were distributed through the circulation at about the same rate, and that the form of the " mixing phase " of the dye curve was primarily due to mixing and not to dye loss

In 2 subjects, Bi and Ro, the agreement between the dilution curves is less good Thus estimates by dye and marked red cell methods of the course of dilution differ by as much as 7% and as much as 9% in some of the samples of subjects Bi and Ro respectively The chief cause of the differences is thought to be technical error The pair of dilution curves shown by Bi are such as might be expected if mixing of marked red cells in total red cells were complete in two or three minutes, but mixing of dye in plasma was either spread over, or dye was being removed during, the first six minutes But the first 4 dyed plasma samples from subject Bi showed appreciable cloudiness varying from sample to sample, so that these estimates of plasma dye content are not precise Ro shows the usual form

of dye dilution curve but an abnormal R B C dilution curve The latter is also thought to be due mainly to technical error, since in this experiment the number of marked to total red cells is about one-half of that in the other
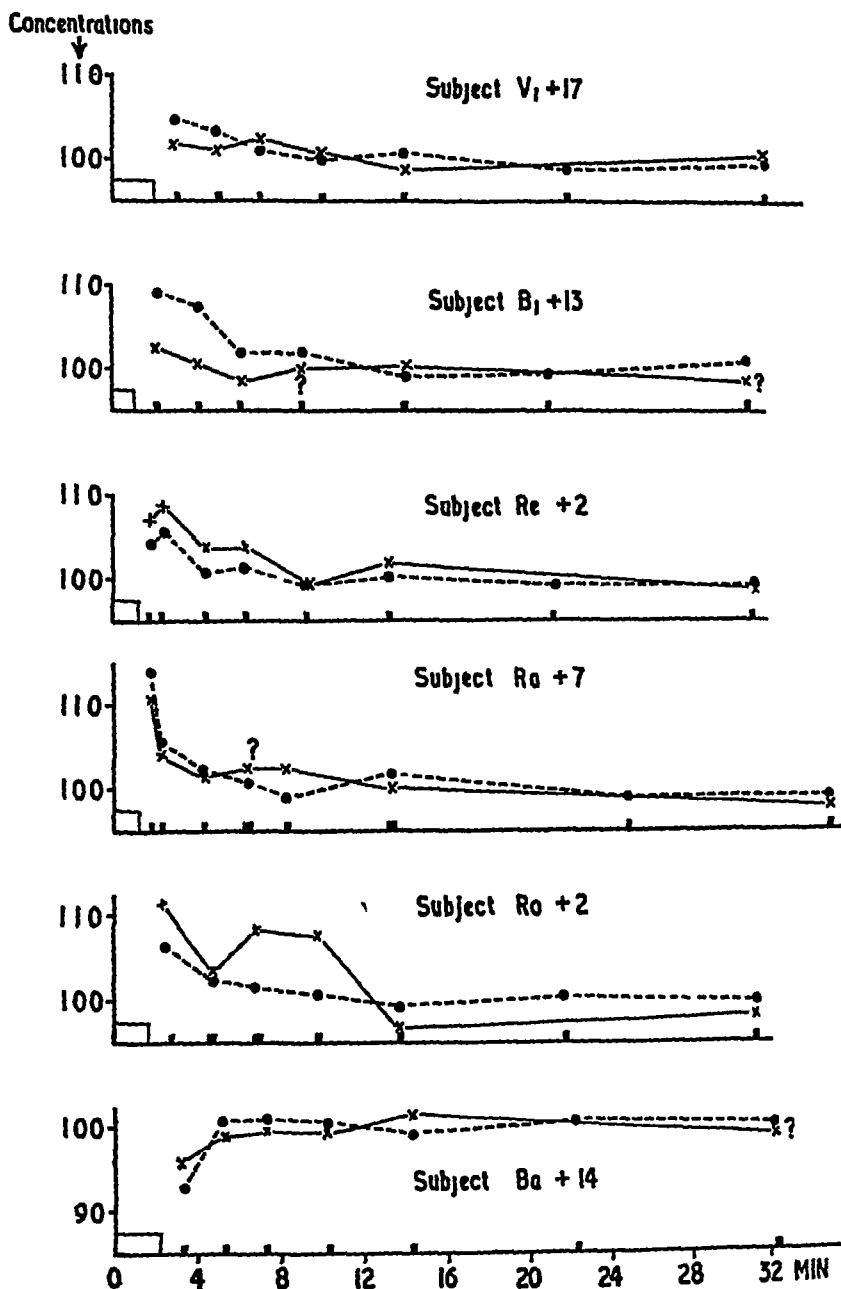
Fig 2   Relative rates of mixing of simultaneously injected T 1824 and marked red cells For method of plotting *see* text

Figures thus $+$ 17 $=$ % overestimate of red cell volume by dye hæmatocrit method

Other symbols as in Fig 1   For meaning of ? *see* appendix

experiments, the experiments *in vitro* quoted in our first paper suggest that the error is greater with higher dilutions of marked red cells

The red cell and dye dilution curves in subject Ba deserve special note Before the transfusion of dye and marked red cells Ba had been bled, and this appears to have altered the shape of the marked red cell dilution curve The shape of the dye dilution curve was also altered in a very similar way The form of the dilution curve of injected marked red cells in the systemic circulation depends on the relative volumes and rates of flow in the series of parallel circuits making up the circulation It is easy to explain the parallelism between the two curves of subject Ba as being due to similar rates of dilution of dye and red cells in the circulation, but difficult to explain it as being on the one hand due to red cell mixing and on the other hand due to dye loss

Though dye loss does not seem, therefore, to account for the shape of part A of the curve, it cannot be said from these experiments that T 1824 is
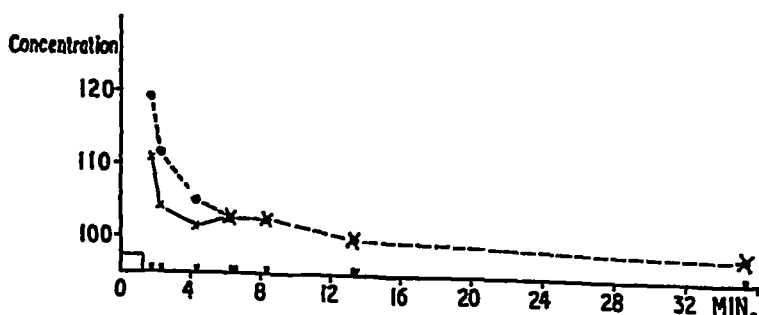
I ig 3   The effect of a 10% dye loss occurring during the first 5 minutes after injection when plotted as the results in Fig 2

Y——Y   Original marked red cell dilution curve of subject Ba

●——●   Calculated marked red cell dilution curve if 10% of the total marked red cells were lost in the first 5 minutes after injection, and the results calculated as in Fig 2   This curve may be taken to represent a dye dilution curve occurring under similar circumstances

not lost during this period   To illustrate this in Fig 3 the marked red cell dilution curve of subject Ba taken from Fig 2 is shown to represent the average course of mixing of marked red cells amongst total red cells   If dye is distributed through plasma at the same rate as marked red cells through total red cells, when plotted as in Fig 2 the dye dilution curve will be identical with the marked red cell dilution curve shown, within the errors of estimation   If now an uncorrected dye loss of 10% of the total dye occurs during the first 5 minutes after injection, then its effect may be determined in terms of the red cell dilution curve   In Fig 3 is shown the red cell dilution curve calculated from the curve Ba of Fig 2 on the assumption that 10% of the total marked red cells was lost during the

first 5 minutes after injection   Taking this calculated curve as representing
the curve found with a 10% uncorrected dye loss and comparing it with
the original marked red cell dilution curve, it can be seen that by the methods
here used it would be very difficult to detect small losses of dye of the order
of *e g* 5% of the total injected

II —THE POSSIBILITY OF DYE LOSS IMMEDIATELY FOLLOWING DYE
INJECTION

Rawson (19) has shown that T 1824 is strongly bound to the plasma
albumin in the concentrations in which its is used for plasma volume
estimations, and this strong fixation is thought to account for its very slow
removal from the plasma   The molecular weights of albumin and dye are
approximately 70,000 and 1,000 so that dye uncombined with protein
might be expected to escape more rapidly from the circulation than dye
combined   In estimating the plasma volume with T 1824, the dye is usually
injected either in solution in distilled water or normal saline   For a time
therefore after this injection, depending on the rate of the reaction between
T 1824 and plasma albumin, there will be uncombined T 1824 molecules in
the circulation which may be expected to escape more readily than those
bound to the albumin   Further it is possible that other proteins, such as
those lining vessel walls, may during this time compete with plasma albumin
for T 1824

Using the red colour filters previously mentioned (1) and 5 or 10 mm
optical cells it is found that solutions of the order of 0 5 to 3 mg T 1824 per
100 ml water or physiological saline have roughly a 10% higher optical
density than the same concentrations of dye dissolved in plasma or serum
This reduction in optical density in plasma or serum solution is due to
combination of T 1824 with albumin (19), and can be prettily demonstrated
by adding traces of pure dialysed albumin solution to solutions of dye in
saline while the optical densities of the latter are being observed in a
photoelectric photometer   The rate of decrease of optical density of a
solution of T 1824 added to plasma or other protein containing solution may
therefore be used as a measure of the rate of combination of dye and
protein   It should be pointed out that the decline in density is not great,
and though sensitive photoelectric apparatus was used, the precision of the
results is only fair

Our experiments were performed in the following way   A known small
volume (1 to 2 ml) of serum or protein containing fluid was placed in an
optical glass cell and on the top of this solution a known small volume
(0 01 to 0 1 ml) of T 1824 dissolved in water, saline or protein containing
fluid was floated   At zero time dye solution and serum or protein containing
solution were rapidly mixed by gentle inversion (10 inversions usually
lasting over 7 to 8 seconds), the glass cell was at once placed in a photoelectric

TABLE

Experiments on the combination of T 1824 and plasma albumin

	A — Effect of temperature on rate of combination of T 1824 and albumin in serum			B — Change in optical densities caused by adding T 1824 mixed with varying amounts of plasma albumin to plasma albumin at 23° C. Final dye albumin molecular ratio of mixtures 1 to 30							
Seconds after addition of solution of T 1824	Temperature			Dye in saline only	40 dye/* 1 alb mol	10 dye/* 1 alb mol	3 dye/* 1 alb mol	2 dye/* 1 alb mol	1 dye/* 1 alb mol	3 dye/* 4 alb mols	1 dye/* 3 alb mols
	0° C	23° C	37° C								
0 10	T 1824 solution mixed with serum for about 10 secs			T 1824 saline and plasma solutions mixed with plasma for about 10 secs							
15	—	100	104	108	108	108	107	106	103	102	—
20	—	107	103	106	107	106	106	105	103	102	101
30	110	105	102	105	105	104	104	103	102	102	101
40	—	101	101	104	104	103	103	102	102	101	100
50	—	103	100	102	103	102	102	102	101	101	100
60	100	102	100,	102	102	102	101	101	101	101	100
75	—	—	—	101	101	101	101	101	101	100	100
90	107	100	100	101	101	101	100	100	100	100	100
120	106	100	100	100	100	100	100	100	100	100	100
3 mins	105	100	100	100	100	100	100	100	100	100	100
5 mins	103	—	—								
15 mins	100	100	100								

The above readings are expressed as % Final Optical Density

The above readings are expressed as % Final Optical Density

* T 1824/albumin molecular ratios of added dye plasma mixtures

photometer, and serial readings of the observed optical density were made for 15 minutes or more The effects of fluctuations in the photoelectric photometer, and alterations in optical density caused by currents in the mixed solution were controlled

In the table are shown a selection of results from a series of experiments in which the observed optical densities are plotted against time Zero time is taken as the start of mixing For simplicity of understanding, though the observed densities have varied between 0 300 and 0 750 and the thicknesses of optical cell between 2 5 and 10 mm the final density reading of the mixed dye and plasma solution, when serial readings have shown that this is constant, is called 100, and earlier readings have been calculated as percentages of this , for instance if the density after 15 seconds is 0 430, and the final density is 0 405, the latter is reported as 100 and the former 106 By this means a general idea of the rates of reactions in different experiments is obtained

In Section A of the table are shown the effects of temperature on the rate of decrease of optical density when small quantities of dye in watery solution are mixed with normal human plasma or serum at different temperatures At a temperature of about 20° C it is seen that the optical density does not reach its final value for from one to two minutes after mixing Lowering the temperature to 0° C considerably slows the rate of this process, though the results shown in the table are only approximate Raising the temperature to 37° C increases the rate of the process, equilibrium being reached 40 to 50 seconds after mixing In these experiments the proportion of albumin to dye molecules varied between 10 to 1 and 100 to 1

To analyse further we have compared the rate of this reaction at room temperature when dye dissolved in saline is added to plasma, with that when dye dissolved in protein containing solutions of varying strength is added to plasma To solutions of 20 mg dye per 100 ml of physiological saline were added varying amounts of human plasma to give albumin/dye ratios varying between 40 to 1 and 1 to 3 These solutions were incubated at 38° C for 45 minutes to allow combination of T 1824 and albumin molecules When cooled to room temperature, 0 1 ml portions were floated on top of 1 0 ml portions of undyed human plasma, the two were mixed, and serial readings were made of the optical density Some of the results are shown in the table, Section B

In the series of results shown the final ratio of albumin to dye molecules was 30 to 1 in all experiments, and hence it may be assumed that the absorption spectrum of the final dye-albumin compound was the same in all the experiments It is clear from the results shown that a change in the absorption spectrum over a period of one to two minutes occurs when mixtures of dye and plasma with dye/albumin ratios varying between 40 to 1 and 2 to 1 are mixed with plasma containing an excess of albumin molecules,

but that as the molecular ratio of the added dye and plasma reaches 1 dye to 1 albumin so the change in the absorption spectrum decreases, and when a ratio of 1 dye to 3 albumin molecules is reached the change is negligible

The change in the absorption spectrum must mean that over the time of the observed change dye molecules are being fixed to albumin molecules It will be noted that when mixtures of dye and plasma in physiological saline with dye/albumin molecular ratios of 1/1 are added to plasma (containing an excess of albumin molecules) there is a small reduction in the optical density This suggests that in such mixtures, as might be expected from the law of mass action, some of the dye molecules are free (or are loosely bound to albumin molecules), but on addition to excess albumin molecules become bound A ratio of 3 molecules of albumin to 1 of dye appears sufficient to bind the major part of the dye, since on adding such a mixture to excess albumin molecules negligible change is observed When mixtures containing 3 or 10 or 40 molecules of dye to 1 molecule of albumin are added to excess albumin in plasma, a greater depression of the optical density is observed, in all three cases as great as that observed when dye in saline is added to plasma Further, when dye albumin mixtures with molecular ratios of 2/1 are added to excess albumin in plasma the depression of optical density is nearly as great Rawson (19) has shown by electrophoresis and ultracentrifugation that in plasma as many as 5 to 8 molecules of dye can be bound by 1 molecule of albumin The above results suggest that in mixtures of plasma and dye in physiological saline with dye/albumin molecular ratios of 2/1 or greater, binding of a proportion of the dye molecules to albumin is not strong, and that on addition of excess albumin molecules there is a rearrangement of dye amongst the albumin molecules to give more stable 1 1 complexes This agrees with general physico-chemical experience, since whether the reaction between dye and albumin be one of chemical combination or physical adsorption it would be expected that the strength of binding would fall off as the number of dye molecules bound by one molecule of albumin increases

In assessing these results it must be noted that the photoelectric photometer in the form used is a crude instrument for such analysis, since it measures the integral light transmitted by the solutions in a band of considerable width, and does not distinguish between a shift in the position of the absorption spectrum and an alteration in the density of absorption Nevertheless these results indicate (1) that an appreciable time is taken for dye added to plasma to become bound to plasma albumin, (2) that when 2 or more dye molecules are bound to 1 molecule of albumin a proportion of these molecules are labile and could presumably be more readily removed from the albumin molecule by a competing protein than when there is a ratio of 1 dye to 3 or more albumin molecules

Practically these results are of importance in two ways

(a) For a short time after an injection of dye for a plasma volume estimation a proportion of the dye molecules may be unbound or labile and therefore more liable to escape from the circulation than firmly bound dye, either by diffusing through semi-permeable membranes, or by becoming fixed to protein other than plasma albumin At body temperature our results suggest that dye molecules are firmly bound within 30 to 40 seconds after injection and hence in such a short period no great loss would be expected from the circulation There is further evidence to support this In the group of experiments reported in Section I of this paper mixtures of dye and whole blood were transfused in which the ratio of molecules of albumin/molecules T 1824 was approximately 3/1 The results in the table show that there is very little optical density change (and hence presumably molecular change) when dye-albumin mixtures with this molecular ratio are added to plasma In our previous paper (1) we reported comparisons of red cell volume estimated by T 1824 and by the Ashby method first in a group of patients, and secondly in the group of normal subjects referred to in Part I of this paper, we also summarized the results of similar observations made by others using other marked red cell methods in normal subjects We have since made further comparisons in normal subjects between the dye estimate of red cell volume and estimates by a radioactive phosphorus method of marking red cells (21) and these confirm the results obtained by the dye and Ashby methods

In all the experiments above mentioned, with the exception of the group recorded in Section I of this paper, dye was injected in water or saline solution Supposing that a considerable proportion of dye escapes when unbound dye is injected into the circulation, and that this escape is prevented by albumin binding, then the group of experiments reported in the first section of this paper should show a significantly higher ratio for

$\dfrac{R\,B\,C\,V \text{ marked red cell}}{R\,B\,C\,V \text{ dye hæmatocrit}}$, called R in our previous paper, than the other

series of experiments The mean of the ratio of our observations on the 8 normals is 0 89 which differs little from the corresponding ratios shown in our previous paper (1), for the results of Gibson and others (10), and Hevesy and others (13) on normals Assuming that the errors of the methods used by Gibson and others (10) and ourselves are of about the same order, a t test may be applied to determine the significance of the difference of the mean calculated for Gibson's figures and for our figures, (8) t is found to be 0 92 for n = 46, which gives a value of P between 0 4 and 0 3, which means that such a difference should be expected in about $\frac{1}{3}$ of such comparisons The difference clearly is not significant

(b) By the time samples of dyed plasma have been drawn from patients and measured, the optical density of the dyed plasma will be stable But in estimating dye standards made up at room temperature in plasma sufficient time must be allowed for the density to reach its equilibrium

value Otherwise erroneous estimates of plasma volume will be made Suppose for instance that a dye standard made up in plasma is measured 30 seconds after mixing, a density 5% too high will be obtained and an estimate of plasma volume 5% too great This effect will be the more noticeable the lower the room temperature because the slower the reaction It will only matter when results of high precision are required It may account for certain results of Chapin and Ross (3) These authors using chiefly the dye method, found that on an average 8 5% of the cell column of the hæmatocrit spun at 1800 × g consisted of trapped plasma These results are about twice the amount of those reported by other authors and ourselves, using rather less centrifugal forces Such results would be obtained if with the dye standard insufficient time was allowed for the reaction between dye and plasma to reach equilibrium and colour filters similar to ours were used, and indeed have been obtained by ourselves on a few occasions before the reaction here described was discovered

III —THEORETICAL EXAMINATION OF THE EXTRAPOLATION METHOD

In this section we examine the question, is it possible by some anomaly of mixing of dye in the circulation or by some defect in the extrapolation method to explain the discrepancy between the estimates of the red cell volume by dye and marked red cell methods It is assumed that the marked red cell estimate is close to the true estimate The estimate of red cell volume by the dye method is determined from the equations

$$(1) \quad \text{Plasma volume} \times \frac{\text{true } \% \text{ hæmatocrit}}{100 - \text{true } \% \text{ hæmatocrit}}$$

the estimate of plasma volume from the equation

$$(2) \quad \frac{\text{Mg dye injected}}{\text{Mg dye found in unit volume plasma}}$$

where the denominator has been corrected for the estimated dye loss by the extrapolation method Hence we are inquiring whether an anomaly of mixing of dye with circulating plasma or a defect in the extrapolation method can cause too low a denominator to be found for this second equation In what follows errors of estimation of the dye content of plasma samples are neglected, for such errors are insufficient to account for the discrepancy, and should in a series of observations cancel out

In only two conditions will an injected substance that is slowly lost from the circulation be unevenly distributed in it if sufficient time is allowed, (a) if a part of the circulation is shut off, so that during the period of sampling the substance does not reach it, or (b) if a part of the concentrated unmixed substance is shut off in a part of the circulation (b) would result in an overestimate of plasma volume, but cannot explain the discrepancies found in the normal reclining subjects described in the first section of this

paper and in our previous paper, since both dye and red cells were injected simultaneously, and either both or neither should be trapped Hence a trapping of dye in one part of the circulation cannot explain a low denominator in equation (2)

With the extrapolation method of correcting for dye loss it is assumed (a) that the true rate of dye loss from the plasma is estimated and (b) that no other dye than that measured as lost escapes from the circulation The loss rate of a dye that slowly escapes from the circulation *and does not return* can be determined from the concentrations of serial samples, provided the dye content of those samples reflects the *mean* concentration of the dye in the plasma at the time those samples were drawn It is clear from experiments such as those of Nylin (15) using marked red cells and our results reported earlier in this paper, that in the circulation of resting recumbent man an injected substance approaches its mean concentration a few minutes after its injection To estimate the rate of dye loss serial samples are drawn during period B of the curve shown in Fig 1, and provided the volume of plasma remains constant (or dye concentrations are corrected to constant volume), and the rate of distribution of dye through the circulation remains rapid, a fair estimate of the rate of removal of a substance that is completely removed from the circulation should be obtained Rather an overestimate of dye loss than an underestimate would be expected from the usual type of curve shown in Fig 1, if dye dilution is incomplete at the time of withdrawal of the earliest samples

The above argument only applies to the estimate of the rate of loss of dye that is completely removed from the circulation never to return, as by reduction to a colourless compound There is a second possible type of dye loss, that in which dye is lost from the plasma *later to be returned* It has been shown that after injections of T 1824 into animals the dye gradually appears in low concentration in thoracic duct lymph (7, 4, 2) If dye passes into the lymphatics and after an interval is returned to the venous circulation, dye is then in effect distributed through a larger volume than the plasma volume For such a mechanism to play a part in causing an overestimate of plasma volume, first there must be a significant amount of plasma dye removed, and secondly there must be a rapid circulation of lymph In fasting, resting, and reclining subjects probably neither occurs Cardozo (2) has reported a greater passage of dye tinged lymph through the thoracic duct than (7) and (4) In anaesthetized dogs with cannulated thoracic ducts the lymph becomes dye tinged 15 to 30 minutes after a dye injection and the concentration of dye gradually rises over the next 2 hours One hour after an injection it may vary in concentration between 5 and 40% of the dye concentration of plasma drawn at the same time Dogs with plasma volumes of between 700 and 1,000 ml under these experimental conditions passed on the average 10 to 15 ml of lymph through their thoracic ducts in 30 minutes Supposing that a total of 50 ml of dye-tinged

lymph is returned to the circulation in the first hour after an injection of dye, having an average dye content of 40% of that of the plasma, in an animal with 1,000 ml of plasma, then an amount of dye equivalent to only 2% of the total in the plasma is returned to the plasma in that time, and can only at most alter the plasma dye concentration by 2% Courtice (4) has made careful experiments and reports much lower values Thus he found that, in 6 dogs a mean value of 0 59%, and in 4 goats a mean value of 0 43%, of the injected T 1824 re-entered the plasma in the thoracic duct during the first hour after an injection of dye In fasting, resting, reclining man it is probable that lymph flow is slow from most sites, and it therefore seems improbable that much dye-tinged lymph will be returned to the circulation in the first hour after an injection Further, if the period in which serial dye samples are drawn is kept relatively short, e g 30 to 40 minutes, there is still less likelihood of re-entry of dye from the lymphatics It is clear however that any circumstances or stimuli that result in rapid lymph flow may result in plasma volume overestimates

It is customary to assume that the rate of dye loss during the " mixing phase " is the same as that estimated after mixing is complete This may not be quite correct During mixing in the circulation an injected substance will tend to be distributed first through the more rapidly flowing circuits Suppose that the circulation consisted of 2 halves of equal volume, and in one half there was a very rapid flow, in the other a slow flow During the earlier part of mixing an injected dye would tend to distribute itself through the more rapidly flowing half, and might approach a concentration twice that of the final mixed concentration Supposing that the removal mechanism is located entirely in the rapidly flowing half, and the rate of removal is proportional to the concentration, then clearly dye would be removed more rapidly during mixing than when mixing is complete This is an extreme example In practice such effects are likely to be small, for the " mixing phase " is short It has also been our custom to inject dye solutions over periods of one to two minutes, which should distribute dye more evenly through the circulating blood and prevent high concentrations persisting

IV —POSSIBLE EFFECTS OF COLOURED IMPURITIES IN INJECTED DYE

It is known that samples of T 1824 may contain impurities (11) and it is theoretically possible that the presence of such impurities might result in the overestimate of red cell volume by the dye method For, if a considerable quantity of coloured impurity that rapidly escapes from the circulation is present, this impurity will be present in the standard made up in vitro but will rapidly escape from the circulation on injection, and therefore will be much diminished or absent in the dyed plasma samples drawn from the subject To be effective such an impurity must have a marked light absorption in the spectral region (near 620 mμ) isolated by

the colour filter of the photoelectric photometer or the slit of the spectrophotometer used for estimation of the optical density of the plasma samples If this impurity is solely responsible for the dye overestimate, from calculations shown in our previous paper (1) it must have an optical density equivalent to about 12% of the total optical density of the injected dye By the capillary test and chromatographic methods we have only been able to detect small amounts of impurity having the necessary spectral properties in 2 samples of dye used in these and other experiments It is possible that our methods have failed to detect this impurity, but it is unlikely that different samples of dye prepared by different manufacturers would contain the same amounts of impurity The agreement between the values reported for $\dfrac{\text{Marked red cell R B C V}}{\text{T 1824 h\ae matocrit R B C V}}$ by ourselves in a previous paper (1), by Gibson and others (10), by Hevesy and others (13) and by another series of comparisons (Reeve and Veall (21)) strongly suggests that the presence of impurities plays only a small part in the overestimate of R B C V by the dye method

SUMMARY

(1) No evidence has been found of dye loss sufficient to explain the discrepancy between T 1824 estimates and marked red cell estimates of red cell volume

(2) Careful comparison of the changes in concentration of marked red cells and T 1824 in the few minutes following the transfusion of mixtures of dye and blood strongly suggests that the " mixing phase " of the dye dilution curve is in fact due to mixing and not to dye loss

(3) At 37°C T 1824 combines rapidly with albumin and it is improbable that much is lost from the circulation before the dye is bound

(4) The extrapolation method ignores two possible types of dye loss but the error so caused is probably very small

(5) Impurities in the injected dye solutions probably at most cause very small errors

(6) It is concluded that the chief cause of the overestimate of the red cell volume given by the dye h\ae matocrit method is unequal distribution of plasma and red cells through the circulating blood

APPENDIX

STATISTICAL METHODS APPLIED TO THE BLOOD COUNTS

For convenience and rapidity of counting, a unit volume of the hæmocytometer chamber was chosen containing on an average 80 to 120 red cells and 9 to 12 such volumes were counted and recorded for each hæmocytometer chamber examined The R.B C counts recorded for the blood transfused to subject Ws are shown in the table To place confidence in a mean derived from such a series of counts it is desirable to have some method for testing for homogeneity, i e of testing if each of the 8 series of counts shown behaves as if it were drawn from the same sample It has been shown that the numbers of red cells of dilute blood samples

STATISTICAL ANALYSIS APPLIED TO BLOOD COUNTS

Subject Ws   The red cells in 12 unit volumes in 8 successive hæmocytometer chamber samples are recorded

Series	1	2	3	4	5	6	7	8	Totals
	102	61	79	74	84	74	79	81	
	107	88	67	100	89	93	85	88	
	98	95	80	86	106	112	93	95	
	97	85	93	88	98	102	72	95	
	108	92	81	100	92	119	78	73	
Counts per unit volume	85	88	101	69	77	96	91	99	
	95	88	81	76	88	78	78	107	
	89	87	84	84	74	85	89	89	
	91	80	112	99	94	98	91	69	
	88	88	104	96	94	88	94	71	
	83	99	86	71	96	92	86	80	
	91	94	82	100	107	75	88	110	
Series Total counts	1134	1045	1050	1043	1099	1112	1024	1057	8564
Degrees of Freedom	11	11	11	11	11	11	11	11	88
Sum of squares of deviations from series mean	733	1015	1763	1613	1117	2151	545	2053	10990

Mean of all counts   = 89 3
Sum of squares of deviations from mean of all
counts   = 11884

ANALYSIS OF VARIANCE

Source	Degrees of Freedom	Sum of Squares	Mean Square	s d
Within series	88	10990	125 (A)	11 2
Between series	7	894	127 (B)	11 3
Total	95	11884	125 (C)	11 2

The 5% point of the variance ratio B/A (*see* Fisher and Yates, Statistical Tables for Biological Agricultural and Medical Research) for degrees of freedom $n_1 = 7$ and $n_2 = 88$ is about 2 2

The s e of the total count 8564 is best estimated from C thus,

$$\pm 96 \times \sqrt{\frac{125}{96}} = 109\ 5 \text{ or } 1\ 28\% \text{ of the total}$$

Assuming the Poisson distribution, the s e of the total count is estimated as $\sqrt{8564} = 1\ 08\%$ of total

are distributed through unit volumes of hæmocytometer chambers as a Poisson series but in the counts shown in the table, and the other counts that we have made, in view of the large number of cells per unit volume and the small number of unit volumes counted per sample it is not possible to apply the usual Poisson technique for estimating the accuracy of the count Since, however, the Poisson distribution approaches closely to the normal for large numbers of cells per square, the counts recorded in each series may be regarded as drawn from a normal population Then an analysis of variance as described in Section 41 of Fisher [8] may be applied This consists in calculating two estimates of the population variance based respectively on the average variation within the series and the variation of the series means and testing whether these two estimates differ significantly by means of Fisher's variance ratio test (8) If they do not then the different series of counts may be considered homogeneous and the total variance of the individual count can be used to give a valid estimate of the standard error of the total count As a check a second estimate of this standard error can be obtained on the assumption that the distribution of individual counts follows the Poisson law, since in that case the standard error of the total count is the square root of the total count The analysis of variance, the application of the z test, the estimation of the standard deviation of the total count, and the value calculated on the assumption that the red cells are distributed as a Poisson distribution, are shown in the table In the example it can be seen that there is no evidence of lack of homogeneity This would be shown if, for instance, the mean square between series was of the order of 300 giving a value greater than 2 2 for the variance ratio The agreement between the s e estimated from the data and from the Poisson distribution is quite good This analysis shows therefore that the samples behave as if all drawn from a single population, and that the counting technique used had a high degree of precision with a standard error of the order of 1% of the mean

Similar statistical analyses have been applied to about half of all counts made for these experiments, and rather shorter analyses to the remainder These analyses show that on the whole the red cell counting technique was good Because of the prolonged mental concentration required in those making the counts, it might be expected that the greater the number of red cells counted per experiment the more likely would errors in the counting technique arise The greatest number of red cells were counted in the experiments on the normals reported here, the total red cells counted per experiment being of the order of 40,000, and in these experiments more errors in counting technique as shown by the above statistical analyses were observed In Fig 2 of this paper each total count which these statistical tests show to be open to greater error is marked with a ? It will be observed that subjects Vi, Re and Ro showed satisfactory counts on all samples, subjects Ra and Ba showed less precise counts on one sample in each experiment, subject Bi showed less precise counts on two samples Hence out of a total of 44 blood samples counted (six samples drawn from the whole blood transfused to these subjects are not shown in the figure but were statistically satisfactory), counts on 4 blood samples only, or about 10% of the total, are shown by analysis to be less precise On analysis, the chief cause of the loss of precision is found to be variation between the means of counts in the series Counts in a series showing a mean differing considerably from the grand mean of all the series might be omitted Since, however, when all the data of our experiments are considered, there have usually only been one or two means out of a total of 6 to 9 in a series in error, since on several occasions a too high mean has been balanced by a too low mean in the same series and since no material difference to the experimental results is made by omitting series of counts showing erroneous means, all values used have been calculated from the total count

The agreement between the s e calculated from the data as described and from the square root of the total count has been good In no case, when the two values have been calculated as coefficients of variation, have they differed by more than ± 0 3%

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CAPILLARY STRENGTH TESTS IN SCURVY AND THEIR REACTIONS TO VITAMIN C AND VITAMIN P THERAPY

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Two types of capillary strength test have been used in nutritional studies In positive pressure tests, stress is applied to the walls of the blood vessels by damming up the venous return from the arm with a sphygmomanometer cuff In negative pressure tests, suction is applied to the skin and this "negative" pressure is at least in some part transmitted to the underlying tissues and blood vessels In both types of test, the ease with which petechial haemorrhages are produced is taken as a measure of the strength of the capillary walls

The application of these tests to human nutritional problems has led to contradictory results (see review (7)) At first it was considered that they could be used for the detection of capillary damage in vitamin C deficiency but subsequent investigation did not always substantiate this claim More recently, it has been suggested that another substance, "vitamin P," may play a part in the nutrition of the capillary walls In view of these conflicting opinions, we thought that it might be of interest to record our experience of capillary strength tests in 15 cases of frank scurvy who were treated with vitamin C and vitamin P Both positive and negative pressure tests were used because, as a previous investigation (4) had shown, the two types of test often give discordant results when applied to the same individual

Methods and Material

Capillary strength tests The positive pressure test chosen was that of Göthlin, as applied by us (3) In this test pressure is applied to the upper arm with a sphygmomanometer cuff and then the petechiæ in a given area

at the elbow are counted   A high petechial count (moio than eight) is considered to indicate impairment of capillary strength   The data reported here are the mean values of determinations made on both arms simultaneously

The negative pressure test used was that of Scarborough (10)   Tests were made on the standard areas of skin defined by Scarborough (area I and area III just below the bend of the elbow, area V at the wrist)   The capillary stiength in each area is indicated by the minimum suction, in mm Hg, needed for the production of one petechial hæmorrhage (critical petechial pressure)   a fall in the critical petechial pressure is taken to indicate a diminution of capillary strength   Both tests weie applied in almost every case, the negative pressure test being applied first

Subjects   These fall into three groups, cases of scurvy and two control groups of nearly the same average age

Group I   This comprised 15 cases of scurvy (mean age 62 years) 14 were males (44 to 81 years) admitted to two local authority hospitals, the remaining case was a woman aged 21 whose diet had been restiicted because of gastritis

Nearly all the male patients had been living for some time in common lodging houses   Their diets consisted of tea, margarine, cheese, sausage and occasionally meat   Eight of the patients had no recollection of having eaten potatoes, vegetables or fruit for at least 9 to 12 months, 6 stated that they took vegetables and potatoes very infrequently   Two had been previously admitted to hospital with scurvy and two other patients were re-admitted with scurvy, one 13 months and the other 24 months after having been saturated with ascorbic acid

The clinical picture presented was typical of scurvy in each case Subcutaneous hæmorrhages occurred on the calves, the backs of the thighs, the dorsal surfaces of the feet, and occasionally on the arms, causing discoloration and induration   About half of the cases showed cutaneous petechiæ, but this was never a prominent feature   Gingival hæmorrhages were found in the few who had teeth   Perifollicular hyperkeratosis was observed in only a small proportion

Hæmatological examinations were carried out to exclude blood diseases known to cause hæmorrhages   All cases were slightly anæmic, but the bleeding and coagulation times were normal and the platelet populations were always well above the critical level for purpura

In all cases the plasma ascorbic acid levels and ascorbic acid saturation requirements were compatible with the diagnosis of scurvy   The mean plasma ascorbic acid value was 0 25 mg per 100 ml plasma (8)   Saturation tests (5) were carried out where the plan of treatment permitted, the criterion of saturation was the excretion of one-third of the test dose in 21

hours (9) The mean quantity required to saturate the patients was 5 6 g (range 2 8 to 9 8 g) Administration of ascorbic acid removed the signs and symptoms of scurvy in all cases

Group II While the scurvy cases were being investigated we examined a group of 29 male patients (mean age 74 years) who had been admitted to the same hospitals with a diagnosis of senility or afebrile chronic bronchitis They belonged to the same social class as the scorbutic cases but ate moderate quantities of potatoes and vegetables

Group III This group comprised 20 healthy elderly men (mean age 65 years) who, while visiting patients in a voluntary hospital, agreed to being tested Questioning revealed that they all ate potatoes and vegetables regularly and fruit when available They were examined in the spring of 1946

Results

Most of the cases of scurvy had been sent into hospital with a diagnosis of " rheumatism " and none had received previous antiscorbutic treatment On admission they were given the hospital diet without potatoes, vegetables, fruit or jam This regimen, by eliminating all sources of vitamin C and probably all those of vitamin P, provided a basic dietary for investigating the effects of vitamin therapy Capillary strength measurements were made before beginning treatment and at approximately weekly intervals thereafter Appropriate statistical tests were applied to the data and probabilities of less than 0 05 have been accepted as significant

TABLE I.

Results obtained with the positive pressure test in the three groups of subjects

Group	No in group	Mean petechial count	Those showing more than 8 petechiæ	χ^2 test
I Cases of scurvy on admission	14	17 30	71%	
II In patient controls	29	7 83	31%	5 22
III Hospital visitors	19	5 03	21%	—

The value of χ^2 has been calculated after making Yates's correction (one of the cells in the fourfold table contains less than 5) The cases of scurvy contain a significantly higher proportion (P between 0 0 and 0 02) of persons showing more than 8 petechiæ than do the in patient controls

Capillary strength on admission Although the mean number of petechiæ obtained with the positive pressure test was greater in the scorbutic than in the non scorbutic patients from the same hospitals (table I), the overlap of results in these two groups was so great that the test cannot be

considered to be of any great value as a diagnostic procedure   McMillan and Inglis (6) came to very much the same conclusion   With the negative pressure test no significant differences were observed between these two groups in any of the three cutaneous areas studied (table II)

TABLE II

Results obtained with the negative pressure test in the three groups of subjects described in the text

Group		No in group	Critical petechial pressure in mm Hg		
			Area I	Area III	Area V
I	Cases of scurvy on admission	14	225 4 ± 100 7	218 5 ± 105 5	362 3 ± 125 5
II	In patient controls	29	205 0 ± 75 0	215 0 ± 77 7	345 2 ± 117 8
III	Hospital visitors	20	222 5 ± 121 0	223 0 ± 116 2	431 5 ± 111 8

Mean and standard deviation are stated for each test

In Areas I and III mean results in groups I, II and III do not differ significantly (Student's t test)

In Area V the mean results in groups II and III differ significantly , t = 2 52, P 0 02 to 0 01   The other differences are not significant (Student's t test)

Since the hospital in-patients (group II) came from a poorly-nourished section of the community we thought it advisable to see whether the tests could differentiate the scurvy cases more sharply from the well-nourished persons of group III, who may be regarded as average healthy members of the population   Examination with the positive pressure test (table I) showed that the hospital visitors (group III) had stronger capillaries than the scurvy cases (group I)   Indeed, the mean number of petechiae declines from group I through group II to group III   Thus the results obtained with the positive pressure test appear to be related to the nutritional status of the group   The results obtained in the three groups with the negative pressure test depended on the area of skin (table II) in the skin areas I and III, all three groups of subjects gave essentially similar results   In area V the capillaries of the hospital visitors were significantly stronger than those of the in-patient controls and were probably (though not significantly) stronger than those of the scurvy cases

Effect of treatment on capillary strength

(A) *Ascorbic acid treatment*   The dose of ascorbic acid varied in individual cases from 200 to 700 mg per day   The average duration of treatment was 20 days (range 6 to 35 days) and the mean dose administered between initial and final readings was 9 6 g ascorbic acid   In every case, the signs and symptoms of scurvy cleared up completely under treatment

Nevertheless of the ten cases of scurvy who had more than 8 petechiæ initially with the positive pressure test, only 3 showed a reduction to less than 8 after treatment with ascorbic acid In the group as a whole, the mean readings obtained with both the positive and negative pressure tests were unchanged by ascorbic acid administration (table III)

TABLE III

Effect of ascorbic acid administration on capillary strength in 10 cases of scurvy

| | Positive pressure test | Negative pressure test | | |
| | Petechial count | Critical petechial pressure in mm Hg | | |
		Area I	Area III	Area V
Mean reading before ascorbic acid administration	18 8	233 9	218 9	333 3
Mean reading after ascorbic acid administration	18 8	196 7	293 3	376 7
Difference	0	37 2	74 4	43 4

None of the differences in the lowest row of this table is statistically significant

(B) *Vitamin P treatment* We administered 4 different preparations known to have vitamin P activity in guinea-pigs (table IV) With the exception of 4 patients who received hesperidin before ascorbic acid, the patients had been given large doses of ascorbic acid for some time Some patients received more than one vitamin P preparation

1 Hesperidin (" Permidin Glaxo ") was given in doses varying from 0 90 to 1 35 g daily These doses appear to be sufficient to strengthen the capillaries when vitamin P is the missing factor (12) The changes in capillary strength observed in our 8 patients on this treatment were probably due to chance Only one patient who had an abnormal result with the positive pressure test (more than 8 petechiæ) before treatment, showed a change to a normal result after treatment (Case No 1) No consistent improvement was observed with the negative pressure test, with the exception of case number 4 where areas I and III showed a marked increase in capillary resistance (area V gave a high reading initially)

2 One rose hip tablet having 275 provisional units (measured by the method of (1) and (2)) was given daily to one case without improvement occurring in either test

3 Orange peel powder was given to three cases at the rate of 10 g daily (approximately 140 mg per kg) This powder was shown to have full activity at a level of 170 mg per kg on deficient guinea-pigs (Bergel,

TABLE IV

Effect of vitamin P preparations on capillary strength in scurvy

Treatment	Duration of treatment with P in days	Daily dose Vit C mg	Daily dose Vit P g	Positive pressure test Petechial count Before	After	Negative pressure test — Critical petechial pressure in mm Hg — Area I Before	After	Area III Before	After	Area V Before	After
Hesperidin	6	—	1 35	17 0	5 5	240	150	150	200	200	300
	7	—	0 90	6 5	5 5	130	170	175	130	300	500
	14	—	1 35	9 0	8 5	350	500	500	500	500	500
	16		0 90	36 5	34 0	180	500	180	340	500	500
Rose hip extract	14	100	0 90	13 0	20 0	100	100	190	100	480	200
	14	100	0 90	22 5	14 0	140	100	390	160	500	500
	14	100	0 90	6 0	11 0	150	190	400	150	300	390
	14	600	1 35	9 0	10 5	350	500	500	500	500	500
Orange peel powder	15	100	—	40 5	26 5	170	90	200	140	350	400
Chalcone	14	10		13 0	11 5	100	140	160	150	480	150
	14	10		22 5	14 5	140	130	350	200	500	200
	14	10		6 0	29 5	150	240	150	350	300	350
	6	0 10	3 40	54 5	15 0	240	110	150	150	170	140
	6	0 10	1 10	29 0	14 0	150	150	200	200	440	500
	6	0 10	1 65	17 5	10 0	300	190	240	350	350	390
	3	0 34	1 35	18 0	15 0	220	100	440	500	500	500
Mean difference			17 3	19 4	171	233	203	243	388	390	
				+ 2 1†	+ 01 3*	− 20 3†	+ 2 5†				

† Difference not statistically significant according to Student's "t" test

* Difference statistically significant according to Student's "t" test   t = 2 28   P = 0 04 approx

If all the data for the negative pressure test in this table are combined the mean difference is + 14 5. This is not statistically significant (P = 0 5 approx.)

personal communication) No consistent changes were observed in capillary strength

4 A synthetic chalcone having full activity in deficient guinea-pigs at a level of 5 mg per kg (Bergel, personal communication) was given in doses of 1 5 to 5 mg per kg daily to four patients In all four the capillary strength deteriorated according to the positive pressure test and improved according to the negative pressure test (areas I and III)

On the whole these sixteen treatments with the vitamin P preparations have not provided any evidence of an improvement in the strength of the capillaries as judged by the results of the positive pressure tests The mean number of petechiæ after treatment is slightly higher than before treatment (table IV) Changes in the results of the negative pressure tests do not occur consistently in all skin areas except perhaps in the case of the synthetic chalcone this treatment, however, caused a worsening in the results of the positive pressure tests It is not possible to say which test gives the better indication as to the strength of the capillaries If we consider the negative pressure results in more detail, we find that in skin area I there is a general tendency for the result to improve after vitamin P and this change is statistically significant In skin areas III and V the mean differences are not significant When the results obtained with each of the preparations of vitamin P are considered separately, statistically significant changes were not found in any of the skin areas with this test If the results for all three areas are combined the mean difference is not significantly different from zero

TABLE V

Capillary strength of 14 subjects admitted with scurvy and dismissed well

	Positive pressure test	Negative pressure test		
	Petechial count	Critical petechial pressure in mm Hg		
		Area I	Area III	Area V
Mean reading on admission	17 30	225 4	218 5	362 3
Mean reading on discharge	18 03	205 4	273 8	410 8
Difference	1 73	20 0	55 3	48 5

None of the differences in the lowest row of this table is statistically significant

(C) *Capillary strength on discharge* Table V shows the results obtained from 14 patients prior to dismissal The capillary strength data are essentially similar to those obtained on admission Thus there was no permanent change in the capillary strength as a result of treatment although these patients were in fact cured

Discussion

With the positive pressure test of Gothlin we have been able to demonstrate a higher petechial count in a group of scurvy cases than in two comparable control groups With the negative pressure test on the other hand, differences between the scurvy cases and the two control groups were not statistically significant although the capillaries in skin area V of the scurvy cases were probably weaker than those of the hospital visitors This want of agreement between the two tests is not surprising in view of the very low correlation between them (4) Analysis of the data in the present investigation in which the readings are distributed over a wider range than was obtained in 1942 shows that there is no correlation between the positive and negative pressure tests in individual cases—they appear to measure quite different properties

It has been suggested (11) that the capillaries of scorbutic subjects suffer from vitamin P deficiency Scarborough claimed that in contrast to the effects of vitamin C therapy administration of vitamin P leads to an improvement of capillary strength and this is taken to indicate a previous need of vitamin P The positive pressure test with which the capillary weakness was demonstrated in some of our cases of scurvy failed to register any improvement after the administration of vitamin P preparations

The negative pressure test gave no indication of a special degree of capillary weakness in untreated scurvy , however, when vitamin P was given, in these cases, there was a significant improvement in one skin area (area I) although an adjacent area of the skin (area III) showed no change Vitamin P does have, therefore, some effect on capillary strength, but it is difficult at this stage to determine its significance The effect does not appear to be lasting and in skin area I the mean readings on discharge were not significantly different from those on admission (table V)

The clinical progress of the scorbutic patients was not reflected in the results obtained with the capillary strength tests Even at the time when the patients were discharged from hospital clinically well, the results of the positive and negative pressure tests were essentially similar to those obtained on admission when the patients were ill and very weak (table V) It seems reasonable to conclude that these tests of capillary strength do not measure the fundamental vascular lesion in scurvy It is, therefore, pertinent to ask why the positive pressure test demonstrates capillary weakness in scurvy if this is not related to the course of the disease As the capillary weakness persists after clinical cure with ascorbic acid and is apparently not due to vitamin P deficiency, we are presented with at least two alternatives, viz , that the capillary weakness was present before the subject developed scurvy, or that the vascular changes associated with scurvy are irreversible In view of the rapidity with which clinical cure can be effected in scurvy, the second alternative seems unlikely to be correct

The other possibility, that scurvy is more liable to develop in subjects who already have some form of capillary weakness, is a more attractive explanation A constitutional capillary weakness is probably present in a small proportion of the population, for, in a group of 346 students studied by the positive pressure method (3), we found 5 with petechial counts which remained persistently high despite administration of ascorbic acid and vitamin P Although this defect is certainly not the result of ascorbic acid deficiency it may make the individual more liable to develop scurvy under adverse nutritional conditions It is interesting to note that a mean petechial count of 18 7 was obtained with the positive pressure test in those of our scurvy cases who needed less than 6 0 g of ascorbic acid for saturation whereas the mean petechial count was only 9 3 in those requiring more than 6 0 g ascorbic acid Although these means are not significantly different, they suggest that individuals with weak capillaries develop scurvy after a smaller deficiency of vitamin C than those with stronger capillaries The recurrence within a relatively short period of scorbutic symptoms in two adequately treated cases seems to fit in with this idea and suggests that the incidence of scurvy in a population may be influenced by factors additional to the deficiency of vitamin C

Summary

1 Measurements of capillary strength have been made by positive and negative pressure methods in three groups of subjects of nearly the same mean age (I) 15 cases of scurvy, (II) 29 in-patients from the same hospitals as the scorbutic cases, (III) 20 well-nourished hospital visitors

2 The positive pressure test showed that the scurvy cases had on the whole the weakest capillaries, but more than a quarter of them gave results within the range found in healthy well-nourished subjects The negative pressure test failed to differentiate between groups (I) and (II)

3 Treatment with vitamin C did not consistently alter the strength of the capillaries as judged by either the positive or negative pressure tests

4 Several preparations said to have vitamin P activity were given to the scorbutic cases with and without previous treatment with ascorbic acid There was no alteration in capillary strength as assessed by the positive pressure test and only a slight increase in strength in one skin area as judged by the negative pressure test

5 Since clinical cure of scurvy can be obtained without any change in the results of the tests, it seems that these tests do not measure the fundamental vascular defect in scurvy

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THE MEASUREMENT OF THE TOTAL LUNG VOLUME AND BREATHING CAPACITY

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THE work to be described in this paper was undertaken as the first stage in an investigation of the physiological mechanism responsible for the breathlessness in coalminers' pneumokoniosis   It has been suggested (13, 14, 17) that focal emphysema, which is a common pathological finding in such cases, might be an important cause of breathlessness , it might lead to impaired lung ventilation associated with change in the relative proportions of the lung volume sub-divisions   We therefore began our investigation of these cases by studying their lung volumes

Various methods were available   From a study of the literature, it was possible to reject many which had proved inaccurate, but it was not possible to decide upon the relative accuracy and simplicity of those most recently described, and our experiments were designed to assess them

Terminology   In this paper we have used the terminology proposed by Christie (4), which is shown diagramatically in Fig 1
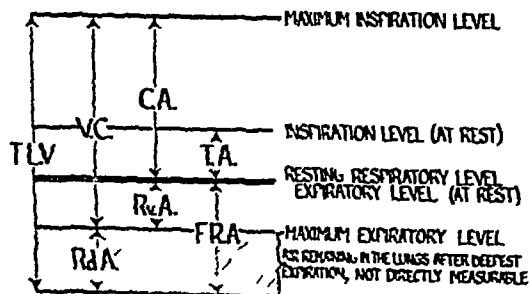

Fig 1   Diagram of the divisions of the total lung volume referred to in this paper   TIV = total lung volume   VC = vital capacity   RdA. = residual air   CA. = complementary air   RvA = reserve air   T.A = tidal air, F.R.A. = functional residual air

It should be noted that in this terminology Complementary Air (C A) is measured from the Resting Respiratory Level (R R L) and so includes the Tidal Air (T A) Functional Residual Air (F R A) is the amount of air remaining in the lungs after a normal expiration

Plan of experiments The paper is divided into four sections following the experimental plan Measurements, which are described in detail below, were made in each of 17 subjects of —

1 *Functional Residual Air* by two methods which are referred to as the " Open-Circuit " and " Closed-Circuit " methods respectively

2 *Vital Capacity* by two methods The first by simple exhalation into a direct reading spirometer (V C simple) the second from the tracing of a Closed-Circuit Spirometer (V C tracing) when the Complementary, and Reserve (Rv A) Airs could be separately recorded

3 *Voluntary Maximum Breathing Capacity (M B C)*

4 *Radiological Chest Volume (R C V)*

The repeatability of a method within a day and from one day to another, as well as the absolute value obtained on different subjects by the various methods, was determined Pilot experiments were first made to achieve technical accuracy with the apparatus and then the main experiment was made in which two subjects were examined each week from May till July, 1947 The main experiment was designed primarily in relation to the measurement of the F R A as this is an indirect determination and one about which considerable disagreement exists

Readings of F R A were taken on pairs of subjects each week Each subject had an estimation by one method only in a day, but the experiments were planned so that on each subject four estimations by both methods were made within the week, two in the morning and two in the afternoon On every occasion estimations were made in duplicate

Directly before a measurement of the F R A on any subject, three tracings of Rv A were made, followed by three tracings of V C from which the components C A and Rv A could be measured Thus, each subject had 12 readings of V C and its sub divisions taken (as three repeat readings on 4 days) within any one week on the closed circuit apparatus Also, six readings of both M B C and V C on the simple spirometer were taken as three repeat readings on the first two days of the week only

The 17 subjects were chosen so as to represent the full range of abnormality likely to be encountered in a group of cases of pneumokoniosis in South Wales coal miners

Subjects were divided into 4 groups according to the radiological appearance of their chests (*see* Table 1)

The first group had no radiological abnormality and had never worked in the coal mines (group N), the second group were working miners who had some minimal dust marking radiologically but who had been rejected by the South Wales Silicosis Board as not being ' certifiable " cases of pneumokoniosis (" Early reticulation " Group E R), the third group had " reticulation " on X ray as described by Hart and Aslett (16) and had been ' certified " with pneumokoniosis (group R), while the remaining group were radiologically grossly abnormal and had massive shadows and evidence of emphysema added to the picture of dust reticulation in their chest (Group G A)

All estimations were made with the subjects in an upright, but comfortable sitting position This position, as Aslett and others (1) remark, has advantages in that it is nearer the position used for taking the chest X ray, allows greater freedom for giving V C measurements, and, in our case was convenient since these subjects were ambulatory

TABLE I

Details of the 17 patients who acted as experimental subjects   The following is the basis of the clinical grading given in the Table —

Grade I — Normal   Not appreciably more breathless than men of same age and build

Grade II — Slight disability   More short of breath on climbing hills than normal men of same age but able to keep up with them on the level

Grade III — Moderate disability   Unable to keep up on the level with men of same age

Grade IV — Seriously disabled   Breathless on the slightest exertion for example crossing the room

Radiological group	Subject No	Clinical grade	Age (yrs)	Ht (ins)	Stem ht (ins)	Chest exp (ins)	Weight (lbs)	Present occupation
1 Normal (N)	1	I	44	68.5	34.5	2.5	133	Prison officer
	4	I	31	65.5	34.5	2.0	151	Male Nurse
	7	I	20	65.5	34.5	1.5	150	Remedial gymnast
	9	I	18	66.5	35.0	2.0	133	Workshop technician
2 Dust mottling but not of certifiable degree (I R)	12	II	32	70.0	36.5	2.0	147	Working "ripper" collier
	14	I	38	70.0	34.0	1.5	180	" "
	15	II	34	67.0	35.0	2.5	155	" "
	16	I	12	61.5	31.5	2.5	140	" "
3 Dust mottling "certified" by Silicosis Board (R)	8	II	30	67.0	35.5	2.0	130	Rat catcher
	10	I	40	65.0	37.5	2.5	165	Colliery surface engine driver
	11	II	53	65.0	35.0	1.5	147	Unemployed
4 Advanced pneumoconiosis with massive shadows and emphysema (G A)	2	IV	50	68.0	35.0	1.0	107	Unemployed
	3	IV	58	65.5	33.0	2.0	130	" "
	5	IV	48	68.5	34.5	2.0	110	" "
	6	IV	44	64.5	34.0	1.0	98	" "
	13	IV	65	63.5	34.0	1.0	100	" "

(Note (a) Subject 13 had no radiological massive shadows but is included in Group 4 on account of his advanced emphysema

(b) In all subsequent tables the radiological groups are abbreviated as follows   N = "normal," E R = "early reticulation," R = "reticulation," G A = "grossly abnormal.")

I   THE MEASUREMENT OF THE FUNCTIONAL RESIDUAL AIR

The Residual Air is the only part of the Total Lung Volume not directly measureable and is derived from a measurement of the Functional Residual Air   Christie (4), in 1932, reviewed no less than 47 papers dealing with different methods available up to that time for measuring Residual Air indirectly   He concluded that the method of Van Slyke and Binger (35) was the most reliable , in this, Davy's (12) principle of gas dilution is used, but the hydrogen employed as the "indicator" gas is used in a closed-circuit spirometer system without forced breathing   Christie emphasized that the Residual Air is best determined by subtracting the Reserve Air from the Functional Residual Air, the latter being measured by the gas dilution   It is now usual to measure Functional Residual Air rather than Residual- Air

In his paper Christie suggested that in order to remove possible danger of hydrogen explosion, the nitrogen already in the subject's lungs could, by itself, be used as the indicator gas if a known volume of oxygen was placed in the spirometer   He realized that this closed-circuit method might suffer from errors from both imperfect gas mixing and from the changing volume of the closed lung-spirometer circuit resulting from the subject's absorption of oxygen during the experiment

Nevertheless, the nitrogen or the hydrogen closed-circuit method remained in general use until it was demonstrated experimentally (25) that large errors did arise from the changing lung-spirometer volume   the end-point of gas dilution was really a state of equilibrium but not with equal gas concentration in the spirometer and the subject's lungs ("nitrogen lag" effect)   Thus, particularly when the subject's Functional Residual Air was large, made the estimate erroneously high

In 1939 Herrald and McMichael (19) published a constant volume modification of Christie's method in which they fed in oxygen at the same rate as the subject absorbed it, and used a katharometer to determine when gas mixing was complete   It was confirmed experimentally (5) that this constant volume modification eliminated the "nitrogen lag" error but the method was still subject to the more serious errors that arise, especially in emphysematous subjects, from incomplete mixing of gases in the lungs

Then Darling and others (10) described an open-circuit method of Functional Residual Air measurement by washing out nitrogen from the lungs   They claimed that this avoided much of the error which may occur during determination of the Functional Residual Air in subjects who have poor pulmonary mixing

Meanwhile, McMichael (29) further modified his closed-circuit method by using hydrogen once more as the indicator gas but in a constant volume system , owing to the relatively high thermal conductivity of hydrogen

he claimed that the katharometer could then be used for the actual gas analysis as well as for determining the end-point of equilibrium In using hydrogen, McMichael incidentally reversed the direction of the indicator gas shift, a procedure which would automatically alter the sign of any error arising from incomplete mixing

These two most recent, but very different methods of estimating Functional Residual Air have never been directly compared

The closed-circuit method has been generally employed in this country and by other European workers (3) and it is important to know whether it does in fact suffer from the mixing errors attributed to it, and, if so, whether the alternative open-circuit method overcomes them The two methods were therefore directly compared in the following experiments, though each was modified in detail to avoid certain technical errors that arise when the apparatus is used in the form described in the literature

Apparatus and method

The closed-circuit method The principle of the method is that when a known quantity of gas is distributed in an increased volume, its concentration will fall in proportion to the increase in volume In practice, a given volume of helium is introduced into a spirometer circuit of known volume containing oxygen The subject is switched into the circuit, breathes from the spirometer for at least 7 minutes, or until equilibrium has been attained, and the combined volume of the lungs plus spirometer circuit is calculated from the observed dilution of the helium

The apparatus and method was that described by McMichael (29) except for the following modifications —

(1) Helium * and not hydrogen, was used as the indicator gas Helium has the advantages that it is not explosive has practically the same thermal conductivity as hydrogen, and has the lowest solubility coefficient in all biological fluids of any inert gas (26) It is not liable to contamination with toxic impurities (The cost of a duplicate F.R.A. estimation with this gas is only 1½d at present day prices)

(2) The katharometer† had four platinum coils in the arms of a simple Wheatstone Bridge, one pair was in contact with the circulating gas, the other pair was sealed in dry air This arrangement give high sensitivity with stability- The gas for the katharometer circulated through a side circuit of the main spirometer system at the rate of 500 c c per minute and reached the elements of the bridge by diffusion, so that readings were not affected by the running of the spirometer pump After a change of gas concentration the katharometer reached a new state of equilibrium in less than one minute

(3) The katharometer was calibrated to record the percentage of helium in oxygen, saturated with water vapour Any other gas e g , nitrogen or CO₂, will also affect the katharometer To overcome such sources of error an extra CO₂ cannister was inserted in the katharom ter side circuit to prevent CO₂ reaching it, should any temporary rise occur in the main circuit An attempt was also made to prevent nitrogen from entering the circuit by having a side tube before the main mouth piece tap from which the subject inhaled oxygen for 15 minutes and so washed out his lung nitrogen, before being switched into the spirometer circuit at the start of an estimation With McMichael's technique where the katharometer records hydrogen percentage in air a small error is introduced by the changing percentage of nitrogen if the lung spirometer volume is not held quite constant during an experiment In our apparatus this error is negligible

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* Supplied by the British Oxygen Company

† Supplied by the Cambridge Scientific Instrument Company

(4) With the apparatus described a subject showing a slow rate of equilibrium on the katharometer in the first estimation can be switched into the circuit filled with pure O_2 for the second estimation before adding the helium It is then immediately apparent whether helium has been completely eliminated from the lungs before starting the duplicate estimation

(5) A fixed orifice flow meter indicating on a sensitive dial reading pressure gauge was used to measure the oxygen in flow when keeping the lung spirometer circuit constant No difficulty was found in keeping the volume constant to within one or two hundred c c in 7 minutes

The open-circuit method The principle of this method is that the nitrogen of the air in the subject's lungs is washed out into a spirometer by successive breaths of oxygen The volume of nitrogen thus eliminated is measured and the lung volume from which it came can then be found The equation used in the calculation of Functional Residual Air by this method is given by Darling, Cournand and Richards (10) and is —

$$\text{Functional Residual Air} = \frac{(V + D\,S)\ (N\,S - N\,O)}{\text{alv } \bar{a} - \text{alv } \bar{p}} - C$$

where V = Volume reading of spirometer, $D\,S$ = dead space of spirometer, $N\,S$ = percentage N_2 in spirometer, $N\,O$ = nitrogen % in cylinder oxygen used, alv $\bar{a}$ = alveolar nitrogen % in patient's lungs at start of experiment, alv $\bar{p}$ = alveolar nitrogen % in sample taken at the end of 7 minutes oxygen breathing, and C = correction for nitrogen excreted from the blood during oxygen breathing

The apparatus and test procedure was similar to that of Cournand and others (6) and is fully described in their paper The only modification, which is described here, is that by which the alveolar samples are collected Figure 2 is a diagram of this modification and is labelled with the same symbols as used by Cournand and others in their paper

It will be seen that the alveolar tubes on the side circuit of the original apparatus have been removed and two alveolar sampling tubes of capillary glass (G) to which Brodie gas sampling bottles (B), are attached, enter the circuit between the subject's mouth and the main tap (V1), through a " Perspex " attachment (P) for the rubber mouth piece (M)

The side circuit ends in a water valve (W) and the rubber mouth piece can be clamped by a large wooden clamp (C) Otherwise the apparatus is unchanged

Before the start of a test, the mercury in each Brodie bottle is pumped up to fill the sampling chamber of the bottle and the sampling tube above it, as seen through the " Perspex " The taps of the Brodie bottles are then closed

The test procedure is then the same as that described by Cournand until at the end of the 7 minute washing out period when the subject is switched from the main to the side circuit by the tap V1, and is asked to exhale completely As soon as he has exhaled completely, bubbling in the water valve (W) stops and the mouth piece clamp is then instantly closed The subject then has to come away from the apparatus, and samples are taken into the Brodie bottles

It is thought that advantage is gained by this method of alveolar sampling as it is fool proof, the smallest leak would immediately be apparent by watching the water level in the water valve during sampling, and, most important, the dead space is reduced to a minimum (less than 50 c c) and samples are taken only 2 inches from the subject's mouth and so represent the last fraction of air from the lungs

Duplicate gas analysis on each Brodie sample was done with a Van Slyke manometric apparatus (32) The mean of duplicate estimations was taken Frequent test estimations of the N_2 percentage in an oxygen cylinder, used as a standard, were made to ensure the accuracy of the gas analysis apparatus, and all agreed within ± 02% of the mean
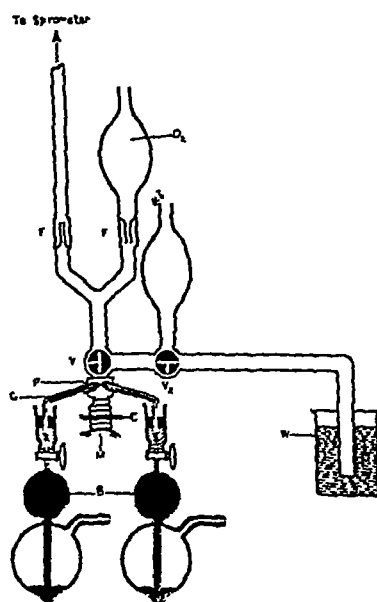
Fig 2   Diagram of alveolar sampling modification to the open circuit apparatus for measuring F R.A described by Cournand and others (6)

B = Brodie sampling bottles, C = wooden mouth piece-clamp, F1, F2 = valves, G = capillary glass sampling tubes, M = mouth piece   P = "Perspex" block, V1, V2 = main and side taps, W = water valve

Results

The results of estimating the Functional Residual Air on the 17 subjects are given in Table II

These results will be considered first in relation to the errors of estimation of each method and secondly in relation to the absolute values found for the Functional Residual Air by each of the two methods in the same subject

The random errors of each method   The duplicate estimations on the different subjects provide data on the experimental errors of each method We consider it better to obtain estimates of experimental error from such data rather than to attempt to obtain it from many estimations on one subject, as each estimation takes about half-an-hour, is tiring for the subject, and his Functional Residual Air itself may change over a long period of time

In recording these duplicate estimations on the subjects, none have been omitted   the blanks that appear in Table II were due to default in subjects or instrumental breakdown

TABLE II

Duplicate Functional Residual Air readings (in litres) by both open and closed circuit methods of estimation

Subject group	Subject No	Day	Open circuit R_1	R_2	Closed circuit R_1	R_2
N	1	1	4 38	4 16	4 41	4 57
		2	4 51	4 03	4 43	4 59
	4	1	*1 77	2 32	3 04	2 98
		2	2 90	2 02	3 12	3 10
	7	1	3 22	3 26	3 01	3 05
		2	2 92	3 06	3 34	3 37
	8	1	3 22	3 39	3 87	3 33
		2	3 41	3 11	3 30	3 11
E R	12	1	*4 45	3 25	—	—
		2	—	—	3 03	3 15
	14	1	4 16	3 53	4 08	4 42
		2	3 08	3 90	3 26	3 50
	15	1	3 45	3 07	4 09	4 20
		2	—	—	4 30	4 41
	16	1	—	—	3 18	3 28
		2	3 04	3 32	3 31	3 00
	17	1	3 38	—	3 47	3 32
		2	3 08	3 26	3 26	3 08
R	9	1	4 85	—	5 35	5 10
		2	3 99	3 94	4 40	4 48
	10	1	2 14	2 10	2 13	2 30
		2	2 26	1 92	1 89	—
	11	1	3 58	3 62	3 82	—
		2	4 75	3 36	4 23	3 88
G A	2	1	2 77	2 89	3 08	3 05
		2	3 05	3 11	—	—
	3	1	3 66	3 32	3 34	2 94
		2	3 48	3 36	3 85	3 27
	5	1	3 65	3 70	3 37	3 31
		2	3 47	3 50	3 28	3 31
	6	1	3 04	2 78	3 09	—
		2	2 87	2 03	2 86	2 71
	13	1	5 30	5 53	6 54	6 42
		2	5 38	5 40	—	—

* These two readings were omitted when computing the error of estimation by the open circuit method as they resulted when gas analysis was known to be faulty

From the differences (d) between pairs of observations, the standard deviation of a difference, about an assumed mean of zero, will be —

$$\sigma^2 d = \frac{\Sigma(d)^2}{N}$$

and hence the standard error of a single observation —

$$\sigma 1 = \frac{\sigma d}{\sqrt{2}}$$

Applying this calculation to the readings in Table II, the standard error of a single determination is found to be 186 c c for the open, and 164 c c for the closed-circuit method   This difference between the random errors of each method is not significant   (Variance ratio at 0 20 sig level)

In making this calculation two readings for the open-circuit method marked with an asterisk in Table II have been omitted, for duplicate analyses on these occasions were unsatisfactory owing to a known fault in the Van Slyke apparatus

A complete analysis of variance on the results would provide an estimate of error, unfortunately the missing readings made this impracticable so the equivalent estimate described above was used   It should be noted that the estimate used, a full discussion of which is given by Dahlberg (8) assumes that the scatter of readings about the mean in all classes of subjects is the same and that the duplicate readings are independent   That the latter assumption is justified in our data was presumed first by the fact that in all cases where repeat readings were obtained the difference between the first and second readings are (within the sampling error) as often positive as negative   and, secondly that in an analysis of variance on the 7 subjects in whom there were no missing readings for either method, the ' between readings " component of variance did not exist significantly in relation to the residual variance   It is of interest that the estimate of error in this analysis on seven of the subjects was 215 and 190 c c respectively for the two methods, and so gives reasonable agreement with the estimate obtained on all subjects by the other analysis

At this stage it is also of interest to compare the repeatability of the two methods used with similar methods employed by previous workers   In doing so the same index of error must be taken   McMichael (29) uses the index we have used and gives SE as 90 c c   Birath's formula (3) is also identical (if d the mean difference, in his formula (q v) is assumed to be zero) and he quotes SE as ± 89 c c for his work in which he uses a similar closed-circuit apparatus for estimating F R A but estimates H₂% manometrically   Christie (4) however, quotes the error of estimate in his work and in his review of that of six previous workers, as the " average deviations from the individual mean "   His figures can be approximately related if it is assumed that the F R A readings are normally distributed when the mean deviation that Christie gives will be approximately 80% of the standard deviation (24)—that is multiplying Christie's figures by the reciprocal of 0 8 makes them comparable (q v)

In over 90% of 158 cases on which duplicate estimations were made by the open-circuit method by Cournand and others (6) the deviation from the mean of a pair was less than 7%   A comparable estimate with the results reported here can be obtained if the mean F.R.A. is as under to be approximately 3 550 c c   7% of this is 200 c c   thus, the range is approximately 400 c c and hence the S D will be of the order of 120 c c , which is in close agreement with our results

It is a fair statement that the standard error of estimation of most methods used to determine F R A up till now has been of the order of 100 to 200 c c

In a more recent series of 47 repeat determinations on 44 consecutive patients performed by us with our closed-circuit apparatus, the standard error of an estimation is 93 c c

The relation between the absolute values given by the two methods   Fig 3 graphically shows this relation   Each point represents the mean value of all readings taken on a patient
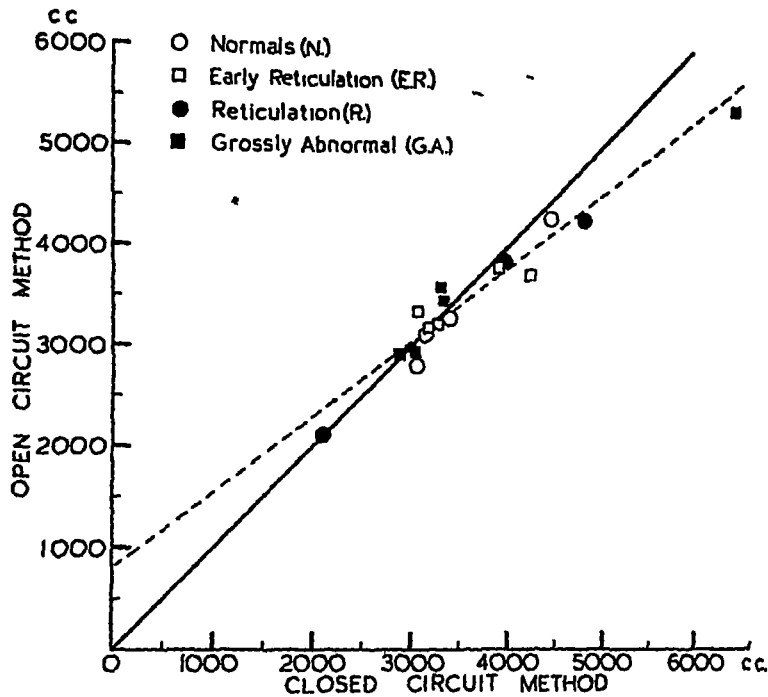
Fig 3   Mean F R A value of each subject as given by the open circuit method plotted against mean value given by closed circuit method   Continuous line is that of perfect agreement between the methods, while broken line is that of " best fit " to the points and is the regression of " open " on " closed "

In Fig 3 the line of perfect agreement and the line of best fit for the points are both shown   We think that the former line is of more interest , it would suggest that there is complete agreement between the methods, within their random error until a subject has a functional residual air greater than about 4 5 litres, after which, the functional residual air appears bigger as estimated by the closed-circuit apparatus

The methods give remarkably accurate agreement and only diverge in recording very large values of the functional residual air   In cases with very large values of functional residual air the precise figure becomes of less practical importance as disability is obvious and day to day variation greater Moreover, although in the extreme cases presented here, the functional residual air is larger as recorded by the closed-circuit method we feel that this is not due to lack of mixing giving a falsely high reading, as Cournand and others (5) have supposed, but that the open-circuit reading is, at these extreme values, itself too low (see discussion)

Discussion

Errors of the methods   Since efforts have been made to estimate residual air or functional residual air in man from 1780 onwards, and as there have always been obvious incompatabilities in the results obtained by different methods, it is interesting that these two very different methods, described here, agree so closely   It is proposed to examine how the results given by these methods are likely to relate to the truth

In estimating the functional residual air in man by gas exchange methods, errors may arise from the following five main causes —

(1) An equilibrium state may be reached between the gases in a lung-spirometer system which is interpreted as equality of gas concentration in all parts of the system which in fact does not exist (*e g*, " nitrogen lag ")

(2) Incomplete mixing of the gases may occur within the lungs themselves

(3) Gas estimation may be at fault, (*a*) chemically, or (*b*) physically

(4) The effect of specific physical properties of the gas used as indicator may not be appreciated, especially in relation to those of the blood

(5) Difficulties may occur in estimating the resting respiratory level from which any measure of functional residual air must, by definition, be made

Obviously, it is not only essential to note which of the above errors arise and their magnitude, but also to consider whether their effect is random or systematic, and, if the latter, in which sense it will then modify the results

The effects of these five error causes on the two methods will be examined

Cause 1 is only applicable to the closed-circuit method   It will occur if the volume of the system is not kept constant   or, alternatively, if inconstancy in the system is not allowed for by alveolar sampling before and after the breathing period (1, 25)   It is a systematic effect and if not removed will make the estimated F R.A too large, especially when the F.R.A itself is big   That its effects can be controlled through keeping the lung spirometer volume constant by finding in oxygen has been shown (25 b)   With our apparatus no difficulty was experienced in keeping the system constant by regulating oxygen flow as measured with the dial type flow meter   Furthermore, with the gas shift from spirometer to lung the necessity of maintaining a constant volume is less important (6) and any error from this cause in the closed-circuit method described will appear as a negligible component *of the random error of estimate*

Cause 2 lack of gas mixing in the lungs might affect either method and will occur especially with emphysematous subjects   If present it may produce an important systematic error   With active distinct in volume and the indicator gas shift from spirometer to lungs, as in our apparatus, the estimated F R A will be too *small* and *vice versa if the gas shift be reversed*

Comroe and others (5) in experiments designed to detect this error by reversing the gas shift claim that the open-circuit method is somewhat less liable to error from this cause though they compared in their experiments a closed-circuit method using nitrogen, not helium, as the indicator gas

Nevertheless, poor lung mixing will affect the alveolar sampling of the open circuit method as suggested from the summary of work on intra pulmonary mixing by Rauwerda (33) The expression used in calculating the F R A in an open circuit experiment, contains in the denominator alv ā — alv p̄, that is, the difference between alv N₂% at the beginning of an experiment and after oxygen breathing for seven minutes Hence errors in either alveolar samples will affect the estimated F R A We agree with Cournand and others (6) that both alv N₂ values might be too low (as measured) when mixing is poor, but errors in their measurement might cancel one another if equal We do not agree that the error in alv p̄ readings is "probably very slight" and hence the F R A possibly too large In normal subjects the alveolar p̄ error may be slight—with the alveolar sampling method used in the experiments reported here, repeat readings of alveolar nitrogen did not differ by more than ±0 2% But in gross emphysema the alveolar nitrogen is high after 7 mins O₂ breathing (in fact it is one index of poor mixing used by Cournand (11)) In addition the reservo air is small compared with the volume of the dead space and hence the alveolar sample is collected and so error may occur In our most severe case of emphysema before the alveolar sample is insufficient to ensure a uniform washing out of the dead space (Subject No 13), the alveolar nitrogen at the end of 7 mins oxygen breathing varied between 13 and 15% on repeat estimations Hence, if the alveolar nitrogen at the beginning of the experiment be assumed, as has been justified (6), the alv ā — alv p̄ expression of the denominator could be too large in emphysema and hence the F R A will be too small The point is debatable

Cause 3   Gas estimation errors are well known   In the open circuit method a range of no greater than ± 0 02% N₂ must be maintained in the Van Slyke analysis to achieve repeatability of F R A comparable to that obtained with a closed circuit estimation   In the closed-circuit method the prevention of N₂ affecting the katharometer eliminates a systematic error likely to make results too large with indicator gas shift from spirometer to lungs, as N₂ has a lower conductivity relative to oxygen than that of helium   The amount of N₂ excreted during a closed circuit estimation, after 10 mins of oxygen breathing is very small and will at the most make closed circuit estimations about 40 c c too large   This error is in opposite sense to any error from unequal mixing

Cause 4   In a closed circuit apparatus with nitrogen as indicator gas, the interchange of the latter with the blood can be a serious source of error   Helium, however, has the advantage of a very low solubility coefficient in water and fat (26)   Using the same assumptions as McMichael (29) but allowing for the lower solubility coefficient of helium as compared with hydrogen we have calculated that the error in the estimated F R A due to solution of helium in the blood is approximately 40 c c and is in the opposite sense to any error arising from incomplete mixing of the gases in the lungs   In the open circuit apparatus a correction has been made for any nitrogen excreted from the blood during the experiment, not as a flat correction, but one that varies with the surface area of the subject (6)

Such a correction may be adequate for normal subjects, but in gross emphysema the nitrogen tension remains high in the lungs during oxygen breathing so that less nitrogen may be excreted from the blood than is allowed for, thus giving F R A values that are too low

Cause 5   The resting respiratory level is inconstant particularly in subjects with emphysema   In the closed circuit methods an estimated mean of a large series of respirations is used to define the level by drawing a straight line which best fits the bottoms of the tidal air excursions   In the open circuit method the subject is switched into the circuit at the end of a particular expiration which may well not lie on the mean R R L   This could be a reason for a larger random error in the open circuit method

Summarizing the above discussion, it is suggested that when the closed-circuit method is used, as described, with helium as indicator going from spirometer to lungs, it is unlikely to suffer from any error greater than that determined as random error of an estimate unless lack of mixing occurs in severe emphysema, when the results as measured might be too small   The open-circuit method also is only likely to be erroneous in emphysema when the correction for nitrogen elimination during the experiment and, possibly errors in alveolar sampling might also give results that are too small

Referring to the graph, Fig 3, the two methods agree and can be presumed to be correct until the functional residual air is greater than about 4 5 litres when those of the closed—are greater than those for the open-circuit In these cases, the closed-circuit estimates could not be too large , can the open-circuit estimate be small to the extent recorded, on the explanations given in the above discussion ?

The correction for nitrogen excretion during 7 min oxygen breathing is of the order of 230 c c , depending on the size of the patient, and error in this correction together with error of alveolar sampling in the same sense might well account for the differences between the two methods, outside the range of their random errors of estimate which themselves may be increased, when the F R A is above 4 litres Bateman (2) has suggested a modification to the open-circuit method to overcome this nitrogen excretion difficulty

It might be argued that circuit resistance would account for the results obtained Actually, the resistance of the open and closed-circuit apparatus was only 2 5 cm and less than 0 5 cm of water, respectively, at 80 litres/min air-flow into the apparatus If there were any effect it would tend to make open results larger than closed, but in fact the effect from such low resistances would be negligible

The conclusion from the direct comparison of open and closed-circuit methods is that these two methods, although very different, give results that are identical for all practical purposes Thus, there seems no reason to abandon the closed-circuit method in favour of the open-circuit if the former be used in the modified form described We, ourselves, prefer the closed-circuit method for functional residual air estimation, in that the random error of an estimation is small, no gas analysis is needed, the apparatus is much more portable, it is economical in personnel, and with it, duplicate estimations of total lung volume can be made within three-quarters of an hour by one person on the one apparatus The advantages of the open-circuit method lie in the information it gives on the effectiveness of intra-pulmonary mixing as indicated by the rate of N_2 elimination and the alveolar N_2 after a period of oxygen breathing (6) Gas elimination curves could be performed on the closed-circuit machine by using a more rapidly responding katharometer

Lung volumes in pneumoconiosis We emphasize that our experiments were designed primarily to test methods and that the findings in relation to subject disability were incidental to this purpose , the value of residual air as a measure of disability, and its relation to other functional tests will be reported later

When from the functional residual air values in Table II, the residual air is calculated by subtracting the appropriate reserve air, and this calculated residual air is expressed as a percentage of the total lung volume,

TABLE III

The residual air (Rd A) of each subject expressed as a percentage of his total lung volume Each figure in the table for either method was obtained by subtraction of the appropriate reserve air (Rv A) value from the mean of all values of the F R A given in Table III The mean alveolar nitrogen value after 7 minutes O_2 breathing, from the open circuit determination of Rd.A is given as it has been used (6) as an index of poor pulmonary emptying especially in emphysema

Subject group	Subject No	Closed circuit Rd A % of T L V	Open circuit Rd A % of T L V	Mean alv N_2% after 7 mins on oxygen
N	1	38	32	1 8
	4	27	26	1 3
	7	21	21	1 3
	8	26	24	1 3
E R	12	32	37	2 9
	14	31	31	2 2
	15	25	22	1 8
	16	30	34	1 3
	17	29	29	1 6
R	9	34	33	1 2
	10	28	28	1 7
	11	46	48	3 0
G A	2	51	53	2 3
	3	53	58	3 4
	5	38	41	2 8
	6	47	48	5 2
	13	59	55	14 2

it is found (Table III) that there is a definite relation, although by no means absolute, between increase in percentage residual air and increase of radiological abnormality This relationship is not seen in the absolute values of residual air, or of functional residual air as given in Table II

These results suggest therefore, that in Coal Miners' Pneumokoniosis the residual air expressed as a percentage of the total lung volume increases with advancing disease and so agrees with similar results in other diseases (21, 23) Moreover, the relation to disability may well be much greater than this table suggests as radiological appearance, while being in general relation, is itself by no means a good index of functional disability

II THE MEASUREMENT OF VITAL CAPACITY AND ITS SUB-DIVISIONS

Vital capacity was first studied in detail in 1846 on a large series of subjects by Hutchinson (22) who defined the term   Since then, many papers have been published in an attempt to establish its " normal " values and their correlation with a variety of anthropometric measurements

In a review of the literature it is surprising to find the scarcity of data on the variation of readings obtained on the same individual at one time, or from day to day, over a short period   With few exceptions repeat determinations have only been made on subjects known to be changing their physiological state, e g , recovering from congestive heart failure (30)

The lack of data arises, in part, from the definition of vital capacity as ' the greatest voluntary expiration following the deepest inspiration " first given by Hutchinson   Most observers have taken three or more observations at one sitting and recorded the largest, others have not stated how many records they took

Peabody and Sturgis (31) in a study of the effects of fatigue on vital capacity showed that 40 observations taken every 15 seconds on 5 patients with cardiac disease did not show a systematic decline over this period Christie (4), who accepted Hutchinson's definition of vital capacity and presumably therefore recorded maximum values, did point out the variation observed in both normal and abnormal subjects when all obvious factors affecting the measurements had been controlled   Cournand and others (8) used a recording spirometer and showed that in normal subjects the vital capacity is ' essentially the same " whether the individual starts with a deep inspiration or expiration, but they did not state if they accepted the mean or maximum figures   Robinson used the maximum of a series of expirations (34)

If the measurement of vital capacity or one of its sub-divisions is to be used as a measure of change of physiological state over a period of months or year it is clearly important to know whether the mean or maximum of a small series of observations made at one time is the more reliable index for the individual   also to know the magnitude of the variation within a day and from day to day under conditions when there is no obvious change in the physiological state   In this series a study was made of these changes in vital capacity

TABLE IV

Vital capacity readings taken on the same subjects by two different methods, the first, simple exhalation into a spirometer, the second from a tracing of a closed circuit spirometer. The variation in V C between normal subjects and those in different stages of pneumoconiosis is shown

Radiological group	Subject No	Day	Simple V C (litres) Reading 1st	2nd	3rd	Mean	Grand mean of groups	Tracing V C (litres) Reading 1st	2nd	3rd	Mean	Grand mean of groups
N	1	1	4 78	4 94	4 89	4 95		4 55	4 80	4 80	4 43	4 88
		2	4 65	4 84	4 73			4 40	4 72	4 30		
		3	4 77	4 53	4 62			4 43	4 25	4 60		
		4	4 50	4 54	4 58			4 28	4 07	3 84		
	4	1	5 40	5 40	5 44	4 74		4 78	4 80	4 01	4 83	
		2	5 23	5 25	5 29			4 83	4 01	4 99		
		3	4 82	4 82	4 82			4 81	4 68	4 70		
		4	4 77	4 80	4 82			4 81	4 88	4 83		
	7	1				5 51		5 38	5 37	5 05	5 40	
		2						5 40	5 48	5 48		
		3						5 45	5 51	5 01		
		4						5 27	5 40	5 40		
	8	1	4 82	4 82	4 82	4 95		4 78	4 83	4 81	4 80	
		2	4 77	4 80	4 82			4 81	4 81	4 91		
		3						4 65	4 75	4 74		
		4						4 74	4 01	4 01		
	12	1	3 03	3 08	3 76	4 0	5 04	3 57	3 03	4 11	3 70	
		2	3 01	3 03	3 84			3 45	3 01	3 65		
P R	14	1	4 05	4 08	4 80	5 05		5 26	5 18	5 15	5 21	
			5 07	5 07	5 13			5 10	4 73	5 35		
								5 30	4 06	5 62		
								5 32	5 28	5 27		
	15	1	5 35	5 21	5 25	5 45		5 13	5 30	5 20	5 12	
		2	5 11	5 18	5 11			5 11	5 44	5 16		
								5 10	5 10			
	16											

Grand mean of all readings

The figures from which they

† These means have been increased by 7% to allow for differences between spirometer calibration are calculated are not corrected as the variability between them is not affected by the error

Christie (4) has pointed out that early evidence of loss of lung elasticity
is obtained by comparison of the vital capacity measurements taken from a
tracing when complementary air is immediately followed by reserve air with
that obtained when there is an interval between the recording of the two
components   In this series reserve air as part of the vital capacity is com-
pared with reserve air taken as a separate tracing

Considering that the manoeuvre of expiring completely is " less natural "
than taking a deep inspiration, it is interesting that Christie found the
average deviation from the individual mean less for reserve air than for
complementary air   However, the values were not expressed as a percentage
of the mean value and thus it was not clear whether complementary air or
reserve air could be recorded with greater precision   We therefore
investigated this point

No examination of the correlation of vital capacity or its sub-divisions
with other anthropometric data is given   The number of subjects was too
small for this purpose and they covered the range from normal to grossly
abnormal   The difficulties of predicting normal values in abnormal subjects
are however discussed later

Apparatus and method

The readings taken by the two methods have been described in the experimental plan

Each spirometer used for V C recording was of 6 litres capacity   The spirometers could
be read to the nearest 10 c c and were arranged so that they were counter balanced throughout
their range of movement , this was achieved by having chains of the correct weight

With both simple and tracings spirometers, each subject was given a trial effort before the
first measurement was made   One to two minutes were allowed between readings   Attention
was also paid to the rate of expiration, as experience showed that a very rapid expiration may
give a figure for the V C which is too low   All subjects were instructed to take at least 5 secs
abnormal subjects, involuntarily, often took very much longer

In calculating the V C volumes from the tracing, the R R L was defined by a line drawn
through the bottoms of the tidal air excursions and the C A and Rv A separately estimated by
measuring the vertical heights of the peaks of respiration above and below this line   In subjects
where the V C took up to a minute or more to complete, the sum of the C.A and Rv A was
greater than the volume obtained by measuring the peak to peak distance off the tracing,
because during the time interval there had been an appreciable uptake of oxygen from the
system, and this sum was accepted as the V C value

All volumes were corrected to 37°C , saturated with water vapour, and at ambient
pressure

Results

Table IV gives the results of recording vital capacity by both simple
and tracing methods on the same subjects   Only the mean value for each
individual and group has been corrected for the 3% difference in spirometer
sizes in this table

The tracing method of recording vital capacity is considered first
The findings from an analysis of variance on the results for all subjects
for whom there are no missing readings by the tracing method, (that is four
normal, three " early reticulation " and " reticulation," and four " grossly
abnormal " subjects) is given in Table V, where mean square figures are
given in deci-litres

TABLE V

Analysis of variance of closed circuit V C readings from Table V for three subjects for whom there were no missing readings (Mean square values in deci-litres)

Group	N		E R		R		G.A	
No of subjects	4		3		3		4	
Source of variation	d f	Mean sq	d f	Mean sq	d.f	Mean sq	d.f	Mean sq
Patients	3	21775	2	17516	2	79976	3	31482
Days	3	787	3	628	3	2650	3	1407
P / D	9 } 39		6 } 30		6	1330	9	1015
Error { Readings R Y D R × P Residue	30	126	24	181	24	207	30	75

From Table V it is found

(1) After allowing for the components of variance in the mean square value of the table, the patient to patient variation accounts for approximately 95% of the total variance in each group, leaving 5% for the error of the estimate

(2) Taking the square root of the estimate of error (times 100 to convert the square to c c), the standard error of one observation lies between 85 and 140 c cs in the different groups That is to say there is an overall repeatability of approximately 100 c c by this method

(3) In groups " reticulation " and " grossly abnormal", the patient-day interaction exists significantly in relation to the error term, that is to say, the day-to-day variation is different between one patient and another These two groups, as might be expected, are not homogenous

Next, in order to compare this tracing-method with the simple-method, it was decided to pool the readings from one day to another, because from the analysis of the tracing vital capacity records (Table IV), it was found that the day to day variation was only 2 5% of the total variance An analysis of the pooled readings is given in Table VI

From Tables IV and VI it is found

(1) There is a significant difference between the two methods

(2) The standard error of one observation (that is the square root of the error term in c cs), is 45 c c and 106 c c for the simple and closed-circuit methods respectively, (note that this estimate of error for the closed-circuit measurements on the pooled data of all patients agrees well with that given by the analysis in Table V) Thus, simple vital capacity is the more repeatable

(3) There is no " learning " or " fatigue " effect with either method, as can be appreciated by studying the grand means of the first, second and third readings on all subjects

(4) If the ratio of the " between-patient " variability to the error term be taken as an index of the sensitivity of a method, simple vital capacity appears more sensitive than tracing vital capacity though both are sensitive tests

TABLE VI

An analysis of variance from all readings of V C in Table V, to compare methods of estimating V C   The analysis was made after pooling the values given on different days by each subject (Mean square values in deci litres)

Source of variation		d f	Mean sq
Between methods		1	2193 0
Method 1 (Simple)	Patients	16	52000 0
	Error	34	20 4
Method 2 (Closed)	Patients	16	45190 0
	Error	34	112 6

It will be noticed in Table IV that in six out of the seven abnormal subjects with a vital capacity of less than 4 litres, the simple vital capacity (corrected means), is smaller than the tracing vital capacity whereas in the remainder of the subjects this is rarely so

A possible explanation, other than chance, is that when using the simple method the abnormal subjects not only find it more difficult to be certain of having taken a full inspiration before inserting the mouthpiece, but cannot hold their breath so long as in the tracing method in which they breathe oxygen between readings

The above statistical analyses are only justifiable if the readings of vital capacity are normally distributed or nearly so   It therefore seemed important to obtain further direct evidence on this point in order to confirm the assumption on which these analyses are made, and to obtain more evidence on the principle of adopting the mean of 3 readings rather than the maximum

To obtain collaborative information one other normal subject recorded his vital capacity in batches of 10 to 12 observations over a period of three weeks until 400 observations had been obtained   Fig 4 is a distribution curve from the results   It is seen that the distribution does not markedly differ from normal   the Beta moments for the date are β_1 = 0 061 (S E 0 081) and β_2 = 3 554 (S E 0 646) and a x^2 test for the goodness of fit to a normal curve gave P = 12
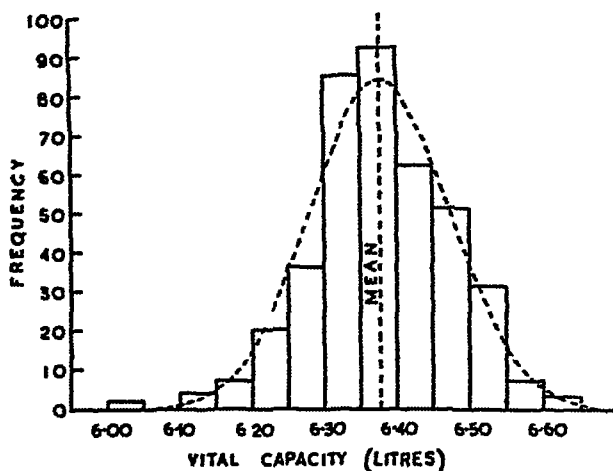
Fig 4 The distribution of 400 readings of the vital capacity of a single normal subject recorded within 3 weeks The normal curve of equal mean and standard deviation is shown by the broken line

It should be mentioned that the daily readings scattered, at random, about a mean which on the whole increased from one day to another as the series progressed The means of the first, second, third and fourth hundred readings were 6 31, 6 36, 6-40, 6-41 Also, it is arguable that this subject had more incentive to give his maximum reading than had the other 17 subjects, and that the normality of distribution cannot be applied to them, but in that case, if low readings were cut out, it would be likely that the scatter of readings, in his case, would be less This was not so the S.D was 94 c c about the mean of 6 38 litres

Hutchinson (22) in 1860 stated that "the vital capacity of man may be considered as a constant quantity" This conception still persists, so that observers assume that the highest value recorded in any one set of readings approaches most closely the true value, in other words, that low readings are due to fatigue or lack of incentive and that the distribution of readings should theoretically be skewed As Gray (15) points out, for maximum breathing capacity, this argument entirely ignores the important fact that random errors of measurement exist and that the mean of a group of figures is a more stable value than a single maximum figure Moreover, the actual vital capacity of an individual is not a constant quantity which is approached but rarely achieved in a series of tests The true vital capacity constantly fluctuates owing to circulatory changes in the lungs, and we consider that the mean of a group of readings is more representative of the vital capacity of a subject at any one time, especially when changes in this value are to be studied over a period

In conclusion it appears that the simple-method is more repeatable than the tracing method, but more sensitive probably because it extends the range of between patient" variation by exaggerating the smallness of the

vital capacity in abnoimal oases　It seems unnecessary to use the tracing method unless the complementary air and reserve air components of vital capacity aie wanted

For the reasons given we have suggested using the mean, rather than the maximum, of a gioup of readings as a figuie to represent the vital capa city of a subject　It is felt that thiee repeat readings are sufficient if their range is not greater than 200 c c　since the repeatability figures show readings difleiing by more than this amount should be very rare in an individual　It may be assumed thet incentive is lacking (which in practice is usually obvious) if the range is greater, in which case more readings are necessary to attain precision for the mean　This procedure is a departure from that usually adopted

On this basis, the vital capacity is re-defined as the mean of at least three readings of the volume of gas expelled by a subject, between maximum inspiration and maximum expiration　This volume should be corrected to 37°C, satuiated with water vapour, at ambient pressure　The subject should not take less than about 5 secs to expel the volume at each observation

Reserve air　Since on each subject three tracings of reserve air preceded three tracings taken as part of a vital capacity whenever functional residual air was recorded by either closed-or open-circuit method, there were on each subject 12 reserve aii estimations separately recorded to compare with 12 estimations recorded as pait of a vital capacity　Allowing for missing readings there were, in all, 380 readings of reserve air

Table VII shows the grand mean reserve air for each group of patients, taken alone and after the deep inspiration as part of a vital capacity recording

TABLE VII

Mean reserve air values on all subjects within each radiological group　For each subject 3 reserve air values were recorded for 4 days　A—recorded separately, B—recorded as part of a vital capacity, that is the exhalation of reserve air was immediately preceded by a full inspiration of the complementary air

Group	A—separately recorded Reading (litres)			B—recorded as part of V C Reading (litres)			Grand mean
	1st	2nd	3rd	1st	2nd	3rd	
N	1 60	1 59	1 56	1 48	1 50	1 54	1 55
E R	1 50	1 50	1 46	1 28	1 31	1 38	1 41
R	1 22	1 32	1 27	1 11	1 13	1 18	1 20
G.A	1 07	1 11	1 15	0 91	0 99	0 98	1 01

Mean of all groups ~ 1 30

In every case, and in all groups, the reserve air alone is greater than reserve air after previous deep inhalation   These differences are barely significant statistically (P between 0 1 and 0 05), but it should be noted that the percentage difference between the two types of reserve air recording is greater in the more abnormal cases   This confirms Christie's finding in abnormal subjects (4) , but obviously the effect may also occur in normal individuals, though less often and to a smaller degree

Complementary air   Table VIII shows the mean of four daily complementary air readings on all patients within a group and the grand means of the groups

<div align="center">TABLE VIII</div>

Mean complementary air of all subjects within each radiological group   In each subject 3 complementary air values were recorded daily for 4 days

Group	Reading (litres)			Grand mean
	1st	2nd	3rd	
A	3 37	3 32	3 38	3 36
1 R	3 31	3 28	3 26	3 28
R	2 58	2 60	2 57	2 58
C A	1 33	1 39	1 33	1 31

<div align="right">Mean of all groups = 2 63</div>

There is no evidence of a systematic change with the order of readings An analysis of variance on the full data of 190 readings gave a standard error of a single determination of 109 c c and this is 0 4 % of the grand mean of all groups

Here again the means of the groups also decline systematically, but more steeply, than in the case of reserve air   The mean of the " grossly abnormal " group is only 39% of the mean of the normals   This agrees with the observations by Hurtado and others (21) that complementary air is more markedly reduced in disease than the reserve air

Though the absolute magnitude of the standard error for complementary air and reserve air is approximately 100 c c , when these are expressed as a percentage of the mean complementary air and reserve air it will be seen that complementary air can be measured with greater percentage accuracy than reserve air

An analysis of variance also showed the patient × day inter-action was significantly greater for reserve air than for complementary air that is, patients showed a greater variation in their reserve airs from day to day than in their complementary air

III —THE MEASUREMENT OF THE VOLUNTARY MAXIMUM
BREATHING CAPACITY

Vital capacity has been regarded as an anatomical or static measure
of lung function (27)   it is not possible for normal subjects to use more
than about 70% of the vital capacity in exhausting exercise   A more
dynamic measure of ventilation was introduced by Hermannsen (18) when
he described the measurement of Maximum Voluntary Hyper-ventilation
Recently Cournand and others (8) have used maximum breathing capacity
extensively but do not give values for the repeatability for the same
individual   They find that maximum breathing capacity correlates well
with the degree of dyspnoea if this is expressed by the formula —

$$\text{Dyspnoeic index} = \frac{\text{Breathing Reserve}}{\text{M B C}} \times 100$$

In this formula Breathing Reserve = M B C — the Ventilation Volume/min
for a given state of physical activity   When the dyspnoeic index falls to
below 60 to 70% dyspnoea is usually present

The voluntary ventilation is used as a measure of a subject's maximum
ability to ventilate the lungs in preference to exercise or raising the inspired
CO_2 for neither of these give values as high as the voluntary hyper-ventilation
Maximum breathing capacity has the additional advantage that it can be
measured with a simple apparatus and also in those who are severely disabled
without causing them undue discomfort   Cournand and others found a
mean value of 154 1 /min (S D 28 3) for the maximum breathing capacity
of normal subjects (8)

Gray and Green (15) in a careful examination of the test on 80 Aviation
Students obtained a mean of 168 1 /min (S D 22)   They showed a signifi-
cant learning effect in a series of three tests made in succession and com-
mented that three tests were apparently insufficient to reach a "plateau"
Despite this, they quoted a high reliability coefficient based on the finding
that the error of the method was small compared with the difference between
subjects

In this series a study was made of the variations of three readings of
maximum breathing capacity at one sitting and between sets of 3 on
different days within a week

Apparatus and method

The M B C is defined as the maximum amount of air that a subject can shift per minute
by voluntary hyperpnoea   This was measured over 15 seconds, it is difficult to maintain a
maximum state for much longer   The mean of a group of readings is taken as representing
the M B C of a subject

The simple V C spirometer was used for measuring M B C   No CO_2 absorbing cannister
was used so that the subject did not suffer from acapnoea as the result of the test   The pulley
wheel of the spirometer was fitted with a series of studs round its periphery so that as the wheel
revolved a series of contacts were made which operated an electrical counter through a relay

TABLE IX

Voluntary maximum breathing capacity of the subjects who are grouped radiologically

Radiological group	Subject No	Day	Reading (litres/minute)			Mean.	Grand mean of group
			1st	2nd	3rd		
\	1	1 2	72 80	72 79	63 80	74 3	122 5
	4	1 2	139 123	144 147	142 115	135	
	7	1 2	84 126	106 106	87 122	105 2	
	8	1 2	147 205	129 205	148 218	175 3	
1 R	12	1 2	81 107	105 113	110 118	105 7	128 9
	14	1 2	100 144	95 124	99 131	115 5	
	15	1 2	126 186	100 192	96 —	140	
	16	1 2	120 —	112 —	108 —	113 3	
	17	1 2	129 126	141 125	138 122	130 2	
R	9	1 2	81 78	81 92	81 84	82 8	79 4
	10	1 2	107 94	115 101	99 117	105 5	
	11	1 2	39 59	43 53	42 64	50	
G \	2	1 2	26 35	26 31	31 34	30 5	30 0
	3	1 2	30 24	24 25	31 27	20 8	
	5	1 2	33 37	31 39	30 32	33 7	
	6	1 2	26 38	29 26	27 30	29 3	
	13	1 2	18 15	22 21	21 23	20	
Grand mean of all readings			85 91	86 48	83 44		

(volume between successive contacts 270 c c) The wheel recorded in both directions The counter was brought into circuit by a switch which was closed synchronously with the starting

of a stop watch   The advantage of recording the movement of the pulley in both directions is that it is then immaterial whether the subject ends at the same phase of respiration as that at which he started   Neglect of this point may produce an error of up to 10%

In addition to recording the volume, a second circuit and counter was arranged so that this circuit was closed while the pulley was moving in one direction only   In this way, a count was made of the number of respirations, thus enabling calculation of the average volume per breath to be made   Results were corrected to 37°C and saturated with water vapour

Special care was taken with this apparatus to keep the resistance to air flow small   2 diameter tubing was used inside the spirometer and for the rubber connection to the metal mouth piece   The importance of maintaining a low resistance was shown by Silverman and others (35) when they observed the maximum minute volume was reduced from 110 to 90 l/min under maximum exercise conditions by a resistance in the circuit of 60 mm H O at 80 l/min flow   The resistance of our apparatus was less than 2 mm H_2O at 100 l/min flow

The subjects were instructed to breathe in and out of the spirometer as deeply as possible, yet as fast as possible   Emphasis was put on depth   An attempt was made to obtain the largest volume by listening to the speed of operation of the relay in the volume counting circuit   This relay operates whenever the pulley moves, whereas the counter does not record until the switch is closed   Thus, during the first 5 secs during which the subject is panting into the spirometer he is instructed to breathe either faster or more deeply until the relay is operating at a maximum rate   Further instructions can be given during the test to keep the rate at a maximum

Results

Table IX gives the results of 98 readings of maximum breathing capacity recorded on 17 subjects   It is seen that the means for groups "normal" and "early reticulation" are approximately the same, but groups "reticulation" and "grossly abnormal" show a marked decline, the mean of group "grossly abnormal" being only 24 5% of group "normal" Thus of all the measures of lung volume made on these subjects, the maximum breathing capacity gave the biggest range of change   The grand means of the first second and third readings on all subjects show no systematic shift An analysis of variance from the maximum breathing capacity results is given in Table X   From this it was found

TABLE X

Analysis of variance from readings of M B C in Table X omitting subject 16 for whom there are missing values

Source of variation		d f	Day 1	Day 2
			Mean sq	Mean sq
Patients		15	5651 0	10,701 0
Error	Readings	2 } 32	01 4	55 5
	Residue	30 }		

1   After allowing for the components of variance the variation between the patients accounted for 97 5% of the total variance and the error of estimate only 2 5%

2   The standard error of a single observation = 7 5 l/min

3   There was no evidence of a learning effect

It is seen that though the range observed in this group of subjects is larger for maximum breathing capacity than for vital capacity or complementary air, the error of a single estimate is also relatively increased so that the percentage of the whole variance due to the error of the method is essentially the same for both maximum breathing capacity and vital capacity Thus, both methods are equally efficient for obtaining a quantitative estimate of the individual, though of course this statement has to be considered in terms of the relationship with other observations of their clinical state

In view of these findings, it is of interest to compare maximum breathing capacity with vital capacity and complementary air for if the correlation is very high, the measurement of maximum breathing capacity may not be worth making The correlation coefficient between maximum breathing capacity and vital capacity is r = + 0 826 and between maximum breathing capacity and complementary air, r = + 0 854 for all subjects

Although these correlations are significant in themselves, they are not significantly different However, it is possible that the closeness of the correlation is principally due to a high correlation between complementary air and vital capacity (r = + 0 89) The partial correlation between maximum breathing capacity and vital capacity with complementary air value held constant is + 0 283 and there is a 15% chance of this being zero The partial correlation between maximum breathing capacity and complementary air with vital capacity held constant is + 0 465 and there is only a 3 5% chance of this being zero Thus, it appears that there is a significantly greater correlation between maximum breathing capacity and complementary air than between maximum breathing capacity and vital capacity This fits in with the finding that the reduction in complementary air is greater than the fall in reserve air in the more seriously disabled groups of subjects However, in neither case is the correlation sufficiently high for useful prediction of maximum breathing capacity from vital capacity

It is a striking feature of those subjects with impaired lung elasticity, bronchial obstruction, or a rigid chest wall, that a vital capacity recording may take up to 60 seconds or more to complete, as compared with the normal 5 to 15 seconds Fig 5 shows tracings from two subjects both with approximately the same vital capacity, but with a maximum breathing capacity of 20 1 /min and 100 1 /min respectively In subjects such as these it is clear that the vital capacity may give a very misleading indication of the ventilatory function of the lung

In conclusion, the above results confirm the work of Gray and Green (15) that the measurement of maximum breathing capacity may be a valuable test of lung function The test is of the same " efficiency " as vital capacity although our patients showed a bigger difference in their maximum breathing capacity with increasing disease than in their vital capacity the random error

of a maximum breathing capacity deteimination was also larger It is a dynamic test and hence is probably more closely related to exercise tolerance than is vital capacity, evidence on this point on a gioup of cases of pneumokoniosis will be published later
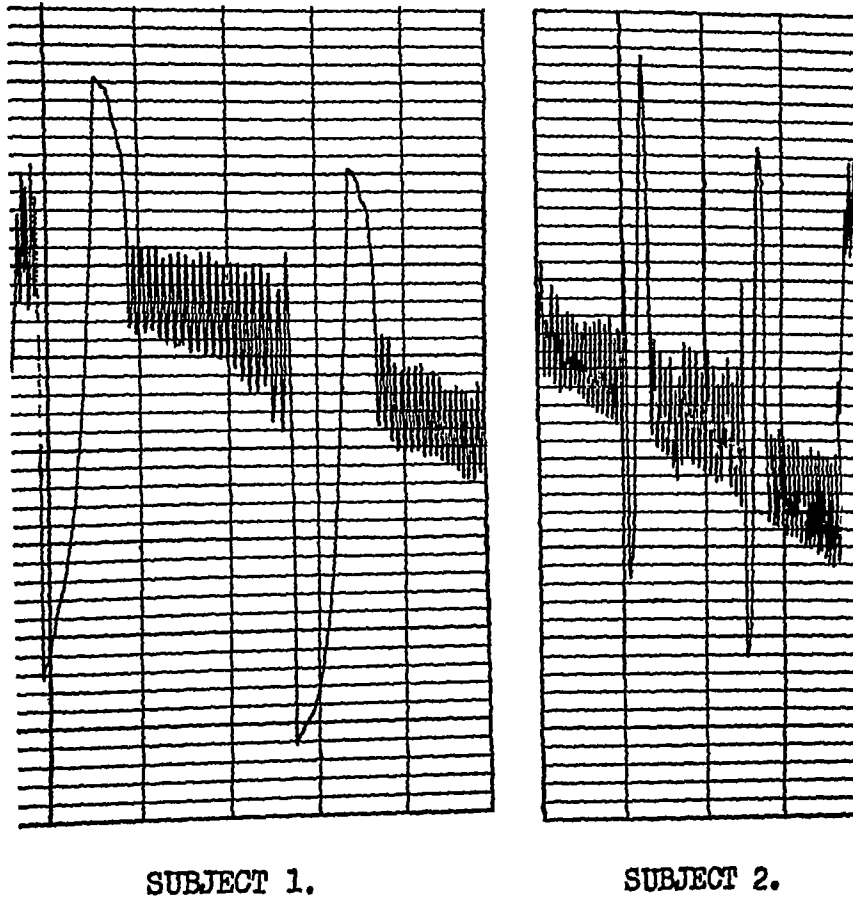
SUBJECT 1.   SUBJECT 2.

Fig 5   Spirometer tracings showing a discrepancy between static ventilatory capacity as measured by V C, and functional capacity as measured by M B C   Each subject has approximately the same vital capacity (3 litres), but Subject 1, with advanced pneumoniosis and some bronchospasm, needs over 1 minute to record this vital capacity so that in exercise only a small proportion is available to him   This is reflected by his maximum voluntary ventilation of only 20 1 per min compared with 100 1 per min in Subject 2 (Horizontal reference lines represent 100 ml, vertical reference lines represent 1 minute intervals)

Like vital capacity, maximum breathing capacity demands co-operation fiom the subject, but in our experience this has not proved a serious difficulty With the apparatus described here, some skill on the part of the observer is requiied to ensure that the optimum rate for a maximum volume is attained The appaiatus is capable of fuither development to reduce the skill needed by the observer, in which case, the random error of the test may be reduced

Maximum breathing capacity is a simple and portable test and causes the patient, even though ill, no discomfort if it is recorded over 15 seconds as described

IV.—THE MEASUREMENT OF THE RADIOLOGICAL CHEST VOLUME

One well recognized limitation to the use of measurements of the sub divisions of the lung volume for assessing disease is the very wide scatter of values in the normal population   Often, in an attempt to overcome this difficulty, the measurements in abnormal subjects are expressed as a percentage of their expected normal value which is predicted from independent physical or radiological measurements

Unfortunately, published work (22, 20, 28) shows that the correlation coefficient of lung volume sub-divisions with anthropometric measurements upon which predicted normal values are based, vary very widely indeed in different groups of subjects, even though age and weight are allowed for, so that it is erroneous to apply a correlation reported for one group of subjects to another   This is a serious limitation

Aslett, Hart and McMichael (1) related the sub-divisions of the lung volume, for a sample of normal South Wales coalminers in whom we are interested, to different anthropometric measurements   They found the highest correlation with total lung volume was that of the radiological chest volume (r = + 0 8) while the highest correlation with vital capacity was that of stem height (r = + 0 78)

In their work they remark on the limited use of prediction formulæ for estimating abnormality in *individuals* owing to the inaccuracy of the predictions   If, however, it were possible more accurately to predict total lung volume, much advantage would be gained as then residual air could be estimated from vital capacity measurements which are easily made on a large scale   In this series of experiments, therefore, an attempt was made to find the limitations of accuracy in measuring the radiological chest volume since this gives the highest correlation with the total lung volume in the South Wales population

Method

Three postero anterior X ray films of each subject in full inspiration were taken with the X ray tube at 6 ft   The A P diameter of the chest was measured by a pelvimeter as described by the previous workers mentioned above   lateral X rays were found to give greater error owing to the difficulty of getting a truly lateral film

The area of the lung fields was measured by a planimeter   the whole area being measured at once by placing the point of the planimeter internal to the area and subtracting the readings from the value for the zero circle of the instrument

In calculating the R C V the regression equation given by Aslett and others (1) was used as in their work was on normals of the same population   The use of this equation was not strictly correct as their V C measurements were not corrected for temperature, moreover, the F R A was measured by the H₂ method,

Results

It was found that by using internal planimetry, as described, the measurement of chest area from the X-ray could easily be repeated to within 2%   That is, error from this measurement is very small

On the other hand, caliper measurement of the chest A P diameter cannot be made with greater accuracy than within 0 5 cm in 25 cm which is the average A P measurement

The most important source of variation between repeat estimations of radiological chest volume in an individual, was found to be due to variation between X-rays—presumably due to the subject not always being at full inspiration

From an analysis of variance of the 48 readings of radiological chest volume in 16 subjects into " between patients " and " between X-rays ", it was found that taking the mean of 3 X-rays gave a standard error of 58 c c in chest volume estimation or, alternatively an error of 71 c c if the mean of only two X-ray volumes is taken   Thus, taking a mean of 2 films gives a radiological estimate comparable in error to that of measuring the total lung volume physiologically *(see* Section 1)

It is felt that it is unlikely that any improvement in the methods of recording total lung volume radiologically or physiologically will lead to a much greater correlation between the two than has been recorded already in the literature

Discussion

In deciding on lung function tests for a group of South Wales coalminers, (for which the work was preliminary) it is possible that re-testing will be done over a long period of time , in this case many lung volume observations such as vital capacity may be of more use than in general medicine, in that subjects will provide their own control and the problem of prediction of " normal " values will not arise

The use of " normal " values for an individual, which are predicted from regression equations determined from a " normal " sample, have been generally unsatisfactory, although much work has been published about the method   One reason for this is that the likely accuracy of the predicted value is so often not stated   It is presumed that if vital capacity is " highly correlated " with another anthropometric measurement, the " normal " value predicted from this measurement will be satisfactory   Actually, the error of the predicted value increases very rapidly indeed as the correlation decreases   This point has been so often overlooked that it is emphasized and illustrated here   The inaccuracy of estimate is a function of the residual variance σ_r, about the regression line and this is $\sqrt{1-r^2}$ times the S D of the dependent variable (24),   This function $\sqrt{1-r^2}$ is plotted against r

in Fig 6 and from this it will be seen how very rapidly the possible range of the predicted normal value increases with decrease in the correlation on which the prediction is placed
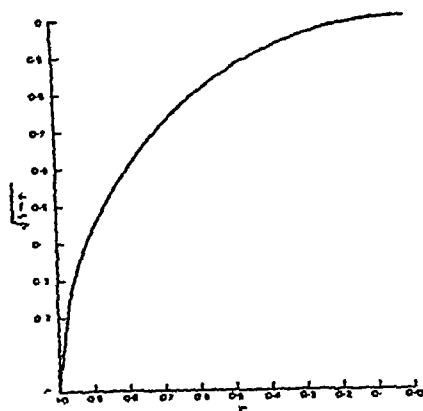
Fig 6 Graph of $\sqrt{1-r^2}$, which is a function of the residual scatter about a regression line, plotted against the correlation coefficient (r) For a given scatter in the dependent variable, the inaccuracy of a predicted normal value for the lung volume of a subject increases rapidly with decrease in the correlation of that lung volume with any anthropometric measurement on which the prediction is based

Thus, any anthropometric measurements are unlikely to be of much use in the prediction of lung volume in *individuals*, unless they are correlated very highly indeed For example, the error of prediction of vital capacity with the highest correlation reported, is such that unless the observed figure differs from the predicted by more than 20%, abnormality cannot be regarded as likely (1, 23)

SUMMARY

1 A detailed comparison of a helium dilution and a nitrogen elimination method for measuring the functional residual air has been made on 13 subjects with pneumokoniosis and 4 normal males The results of the two methods agreed within their random errors (S E approx 100 c c) up to an functional residual air of 4 5 litres The helium dilution method is preferred

2 Vital capacity can be measured at least as accurately with a simple spirometer as from a spirogram (S E approx 100 c c) Evidence from 289 readings on 17 subjects and 400 readings on one other subject lead to the re-definition of vital capacity as the mean in place of the maximum of a series of observations Complementary air can be measured with greater percentage accuracy than reserve air and showed greater change with increase of pneumokoniosis

3 The use of radiological chest volume for predicting "normal" values is discussed, and data given for the accuracy of its estimation

4 Maximum breathing capacity (S E approx 8 litres per min)
showed a greater degree of change than vital capacity in pneumokoniosis,
but both maximum breathing capacity and vital capacity are equally
" sensitive " tests.

5 The experiments suggest that vital capacity, maximum breathing
capacity and residual air as a % of total lung volume may be useful
indices of disturbed lung function in coalminers' pneumokoniosis

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THE LABILE NEUROGENIC COMPONENT OF HYPERTENSION

A COMPARISON OF THE EFFECTS OF TETRA-ETHYL-AMMONIUM BROMIDE AND A RAPIDLY ACTING BARBITURATE (SECONAL)

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THE blood pressure of hypertensive patients shows wide variations as a result of external stimuli and emotional disturbances This lability has long been recognised (3, 4, 10), and wide use is made of the terms " casual " and " basal " blood pressure , the former signifying a chance or initial reading, while the latter implies a reading taken under basal conditions There is no standard method of recording the blood pressure of hypertensive patients, and, in the absence of details of the lability of the pressure in individual patients, it is difficult to interpret or compare many clinical reports on hypertension

The Joint Report of the Committee appointed by the Cardiac Society of Great Britain and the American Heart Association in 1939 recommended that the basal blood pressure should be determined under the conditions used for estimation of the basal metabolic rate Alam and Smirk (2) recorded the blood pressures of patients sitting at ease Hammarström (10) prefers to take two-hourly readings throughout the twenty-four hours, in the resting patient Sedation with barbiturates produces a basal reading comparable to that obtaining in normal sleep, and the Amytal test, introduced in 1936 (8), is widely used in the investigation of hypertension Tetra-ethyl ammonium bromide (T E A B) has recently been shown to produce a fall of blood pressure in hypertensive patients (15), and the results obtained with T E A B and with Amytal have been compared in assessing patients prior to splanchnicectomy (7, 11, 15)

It is now generally accepted that elevation of the diastolic pressure is of greater clinical significance than is that of the systolic The diastolic pressure approximates to the mean pressure throughout the cardiac cycle and is therefore a better indication of the strain to which the vascular bed is subjected strain which is believed to be responsible for secondary arteriolar damage especially in the cardiac cerebral and renal circulations

In patients with hypertension, lability is as much a feature of the diastolic as it is of the systolic pressure, even though the absolute pressure changes are less, and a record of the basal diastolic pressure provides a better measure, than does a casual reading, of the continuous strain on the arteriolar bed

The basal diastolic pressure is likely, therefore, to be of prognostic value, but it may have further significance   Hammarström (10) has shown that the lowest pressure recorded under the influence of Amytal is identical with the lowest reading obtained during twenty-four hours' rest   If further evidence can be provided to show that, using different tests, identical readings of minimum pressure are obtained, there will be reasonable grounds for assuming, not only that these various methods remove the same element in hypertension, but that the remaining or basal pressure is of real significance, and that it is probably dependent upon a different mechanism

This paper presents a comparison of the minimum diastolic pressure readings obtained in a series of hypertensive patients by the use of tetra-ethyl-ammonium bromide (T E A B) and Seconal (Sodium propyl methyl carbinyl allyl barbiturate), and is not concerned with the value of either drug in assessing the suitability of cases of hypertension for splanchnicectomy Seconal, which has a short duration of action, was selected in preference to Amytal because it is more rapidly effective

METHODS

(a) *Patients investigated*   Forty-one patients, found to have hypertension on admission to hospital, were investigated with both T E A B and Seconal   30 cases of benign essential hypertension, 5 of malignant hypertension, 5 of chronic renal disease and 1 case of acute nephritis   Two patients with benign hypertension failed to fall asleep with Seconal, while one case of malignant hypertension had a severe reaction following T E A B The response of the remaining 38 patients to both drugs is analysed   Other hypertensive patients have also been investigated with one or other agent

(b) *Blood pressure records*   The blood pressure was usually measured with an aneroid sphygmomanometer, which was frequently checked against a mercury column   The diastolic pressure was recorded at the level of the disappearance of the sounds, since Steele (18) has shown that this corresponds more closely with the true diastolic pressure recorded by intra-arterial manometry   The following terms are used in this paper —

(i) *Initial blood pressure*   This is the first pressure recorded at the start of a series of readings   It varies in the individual patient with previous physical and mental activity and may show considerable variation from day to day

(ii) *Resting blood pressure*   This is the pressure taken with the patient resting under basal conditions and was measured, in most patients, prior to the administration of T E A B

(iii) *Minimum diastolic pressure* (M D P)   The minimum diastolic pressure with either T E A B   or Seconal represents the lowest diastolic pressure recorded while the patient was under the influence of the drug

(c) *Tetra-ethyl-ammonium bromide* (T E A B)   With the patient recumbent in a quiet room, the blood pressure was determined at five minute intervals until a resting level was reached   The T E A B was then injected into the antecubital vein of one arm, while the blood pressure was recorded at half-minute intervals in the other, until the minimum reading was passed This usually occurred within five minutes and the blood pressure was then recorded at minute intervals

The T E A B , in a solution containing 100 mgm per c c , was injected over a period of 30 seconds   The usual dose was 500 mgm   It has been shown that the average fall in pressure does not increase with doses above this level (7)   In a few frail or ill patients a smaller dose has been used Only one untoward effect was observed   The blood pressure of a man, aged 54, with malignant hypertension, fell to below 60/50 mm.Hg with signs of cerebral anoxia   This alarming reaction, responding immediately to the injection of adrenalin, has been described in similar cases (14) (15), and has been regarded as a toxic effect (6)   Adrenalin was always kept ready for use in the event of similar reactions

The minimum diastolic pressure recorded was taken as a measure of the effect of the T E A B   This minimum level is transitory   The subsequent rise may be due to compensatory mechanisms initiated by the fall in pressure, for in patients with pheochromocytomata, the injection of T E A B results in a marked rise of blood pressure, presumably due to an excessive liberation of adrenalin (12)

(d) *Seconal*   With the patient in his own bed, in a quiet room, the initial pressure was recorded and the Seconal given with a draught of water The blood pressure was determined at five minute intervals until a level base line was obtained or the patient roused   The minimum diastolic pressure recorded was taken as a measure of the effect of the drug   (The minimum pressure is occasionally transitory, and little reliance can be placed on only one or two readings taken while the patient appears asleep)

A dose of 4½ grains usually produced deep sleep, but in 5 patients this dose was inadequate and to these 6 grains were given on another occasion This amount failed to produce sleep in two patients   In frail or ill patients, 3 grains were often sufficient

RESULTS

(a) *T E A B*   The general effects observed following the intravenous injection of T E A B were identical with those previously reported by other workers (14) (15)

Four typical records of the diastolic pressure following T E A B are shown in Fig 1   In normal subjects (Curve 1A), and in hypertensives whose blood pressure falls to normal with rest alone, (Curve 1B), little, if any,

fall below the resting level occurs   In patients with a moderate degree of hypertension, with a raised resting diastolic pressure of 90-110 mm Hg, a considerable fall is usually obtained   In hypertensive patients with a high initial diastolic pressure the type of response varies   In some there is a large fall of blood pressure (Curve 1c), whilst in others the fall is small (Curve 1d)
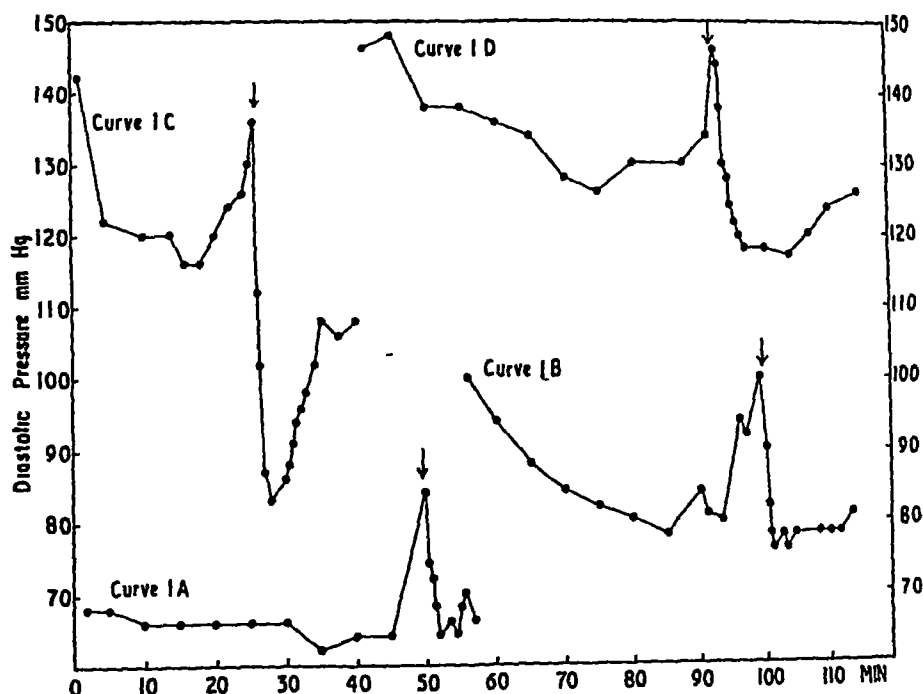
Fig. 1   Four typical curves of diastolic pressure showing fall to resting level and response to T E A B   T E A B injection shown by arrows   Note " Needle Response " in each case

Lyons and his co-workers (14) maintain that the extent of the decrease in diastolic pressure is largely dependent on the initial elevation, but, in our experience, it is impossible to predict the extent of fall in patients with an elevated initial or resting diastolic pressure

The following table of the average and percentage falls in relation to the initial diastolic pressure, emphasises the wide variability of response

Initial Diastolic Pressure	No of Patients	Average fall Diast Press mm Hg	Range mm Hg	Average % fall Diast Press	Range % Fall
90 109 mm Hg	14	14 3 mm Hg	+ 8 to —45	14 6%	+ 9% to —47%
110 129 mm Hg	13	31 6 mm.Hg	—13 6 to —65	26 6%	—11% to —57%
130 mm.Hg and over	21	30 3 mm Hg	— 6 to —48	21 0%	—5% to —36%

In all patients, whether hypertensive or not, a sharp rise in both systolic and diastolic pressures accompanies the application of the tourniquet and insertion of the needle   This "needle response," which is shown in all curves in Fig 1, appears to be emotional in origin   It has been ignored by other observers and may explain the occasional failure of the diastolic pressure to fall after the injection   Apprehensive patients, too, may not respond with a fall and may even show a rise of pressure above their resting level   This is probably due to an excessive release of adrenalin which has been shown (1) to abolish the depressor action of T E A B
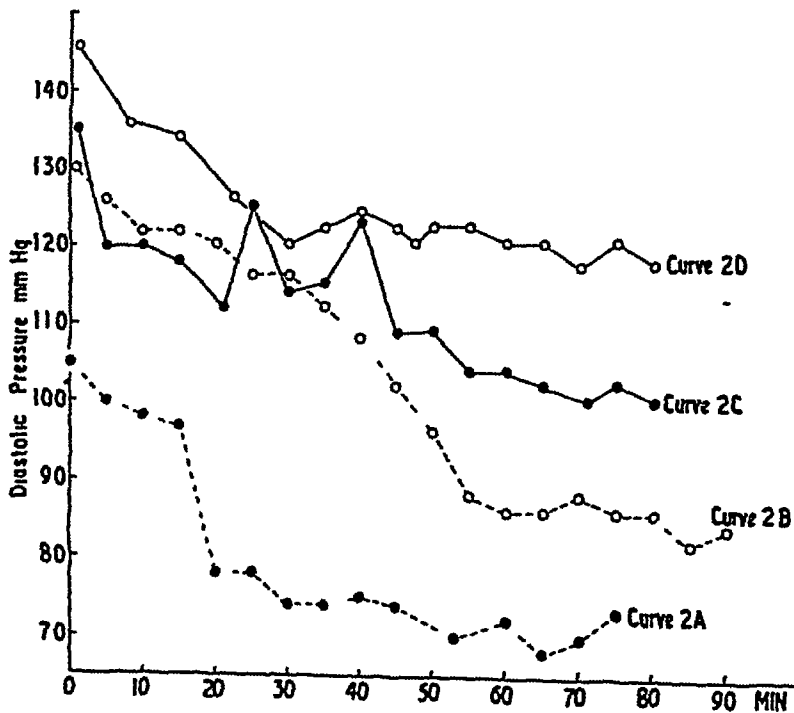
Fig 2   Four typical curves of diastolic pressure following Seconal   In each case 4½ grains of Seconal were given by mouth after the initial reading

(b) *Seconal*   The patient is usually asleep within 15-30 minutes, and the minimum diastolic pressure obtained within 90 minutes   Fig 2 shows four typical responses of the diastolic pressure to Seconal   As with T E A B, the extent of the fall of blood pressure could not be predicted from the height of the initial pressure   The average and percentage falls in relation to the initial diastolic pressure are shown in the following table which emphasises the wide variation in response

Initial Diastolic Pressure	No of Patients	Average fall Diast Press mm.Hg	Range mm Hg	Average % fall Diast Press	Range % Fall
90 109 mm Hg	11	16 6 mm Hg	— 4 to —29	20 6%	—4% to —35%
110 129 mm.Hg	15	25 6 mm Hg	—10 to —42	20 1%	—8% to —36%
130 mm Hg and over	19	30 3 mm Hg	—10 to —48	19 6%	—7% to —37%

The depth of hypnosis is not great, for, even though Cheyne Stokes respiration has occasionally been observed, the patients can at all stages be readily roused   Occasional patients have complained of disturbing dreams, which may account for a failure to secure a maximum fall   MacWilliam (10) noted considerable variation in blood pressure during disturbed normal sleep, and ascribed this to dreams and nightmares   Occasional patients do not fall asleep quickly and, in these, the fall of blood pressure is delayed   Two patients failed to sleep even with a dose of six grams

The discomfort of a full bladder will maintain the blood pressure at high levels despite drowsiness, and, in these cases, the pressure falls rapidly once the bladder has been emptied   The pressor effect of a full bladder has been observed in patients with spinal cord lesions above the level of T 5 (19), and has been reported in cases of essential hypertension (5)   It has also been observed by us to prevent a fall of pressure to a true resting level prior to the injection of T E A B , but this pressor effect is abolished by T E A B

(c) *Comparison of T E A B and Seconal*   With either drug there is no relation between the initial and minimum diastolic pressures , for a given diastolic pressure, the fall that may follow the administration of either drug cannot be predicted   It is of all the more importance, therefore, that the minimum diastolic pressures obtained in any individual patient by the two methods are closely similar

In Fig 3 the minimum diastolic pressure (M D P) obtained with Seconal is plotted against that obtained with T E A B   The correlation is obviously high   Fig 4 shows the distribution of the differences in M D P's obtained by the two methods

Of 38 cases tested with these two drugs —

11 showed a difference of 2 mm Hg or less between the two M D P's

21 showed a difference of 6 mm Hg or less between the two M D P's

29 showed a difference of 10 mm Hg or less between the two M D P's

In four cases there was a difference of 20 mm Hg or more between the two M D P's   Two cases showed a drop to 78/50 and 70/50 mm Hg with T E A B , while with Seconal they fell only to 106/70 and 110/76 mm Hg

In the first case, the minimum pressure obtained with T E A B on another day was 122/72, while in the second case, T E A B given during Seconal hypnosis led to a fall to 80/56 mm Hg
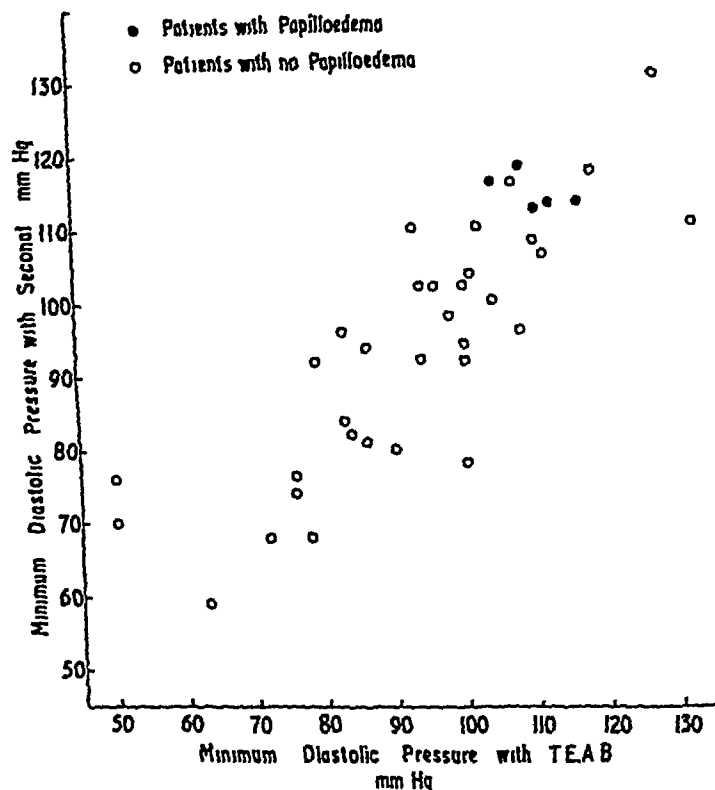
Fig 7   Comparison of minimum diastolic pressures obtained in 38 hypertensive patients using Seconal and T E A B

In the other two cases the Seconal response was greater than that obtained with T E A B, a difference probably due to emotional factors In one the resting diastolic pressure of 76 mm Hg rose to 100 mm Hg with T E A B while in the other difficulty was encountered with the injection

In seven patients T E A B was injected during Seconal hypnosis In only one case (of malignant hypertension) was an M D P recorded considerably lower than that obtained with either drug alone

The correlation can well be seen in the following short case reports, which have the advantage of emphasising that the M D P is independent of the initial diastolic pressure
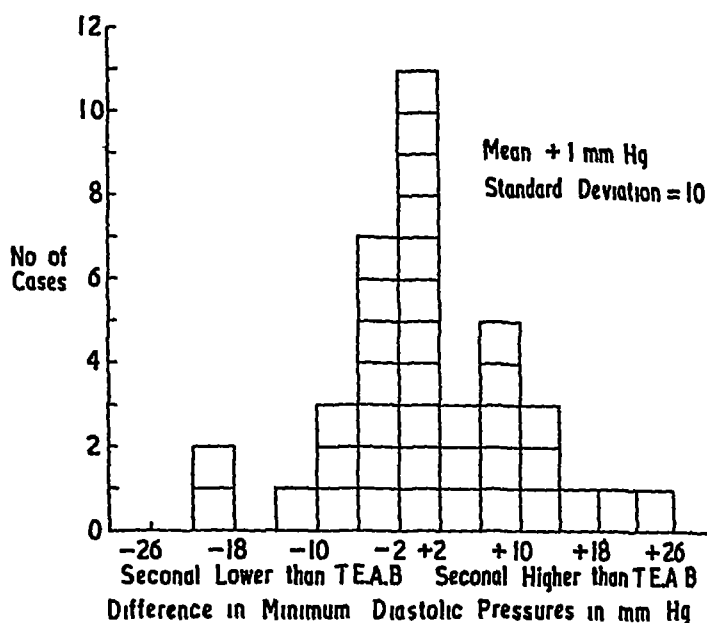
Fig 4   Distribution of differences in minimum diastolic pressures obtained with Seconal and TEAB

Case 1   C G , a housewife aged 37, was admitted on 26th July, 1947, complaining of severe occipital headaches, giddiness, breathlessness on exertion, pain in the left shoulder and nocturnal frequency of micturition for three years   Examination showed a blood pressure of 208/132 mm Hg , enlargement of the left ventricle, retinal changes of moderate severity, but normal renal function

    5th August, 1947   Initial B P 208/132 mm Hg
                     After T.E A B 114/84

    7th August, 1947   Initial B P 206/130 mm Hg
                     After Seconal 114/82

Case 2   E H , a stenographer aged 64, was first admitted on 20th November, 1944, complaining of tiredness for 8 months, breathlessness and palpitations for 5 months and unsteadiness on her legs for 1 month   Her blood pressure was 238/130 mm Hg , her fundi showed early arteriosclerotic changes, there was no cardiac enlargement and the renal function tests were normal   She was treated with potassium thiocyanate, and has remained under regular supervision since, with complaints of precordial discomfort and giddiness on effort Her systolic pressure fluctuated between 210 and 260 mm Hg , and her diastolic between 128 and 150 mm Hg   She was re admitted to hospital on 11th October, 1947, when her blood pressure was 210/140 mm Hg , her fundi showed more advanced changes with a retinal venous thrombosis, and there was slight impairment of renal function

    14th October, 1947   Initial B P 216/128
                        After T.E.A B 140/86

    16th October, 1947   Initial B P 264/124
                        After Seconal 146/94

(The curves in this case are shown in Fig 5)

Case 3   K B , a housewife aged 51, was admitted on 5th October, 1947, complaining of headaches for 7 weeks, visual disturbance and giddiness for 5 weeks and dyspnœa on exertion for 3 weeks   Her blood pressure was 205/110 mm Hg , her heart was not enlarged, her fundi showed early changes and her renal function tests were normal

    14th October, 1947   Initial B P 224/136
                        After T E A B 154/98

    16th October, 1947   Initial B P 226/132
                        After Seconal 146/98

Case 4 C.B , an iron founder aged 42, was admitted on 6th October, 1947, complaining of violent right sided parietal headaches for 6 months His blood pressure was 260/140 mm Hg his brachial arteries were tortuous and his left ventricle enlarged A chest X ray showed early pneumoconiosis His fundi showed early changes and his renal function tests were normal

9th October, 1947   Initial B P 218/140
                 After T.E.A B 134/110

11th October, 1947   Initial B P 240/135
                 After Seconal 146/108
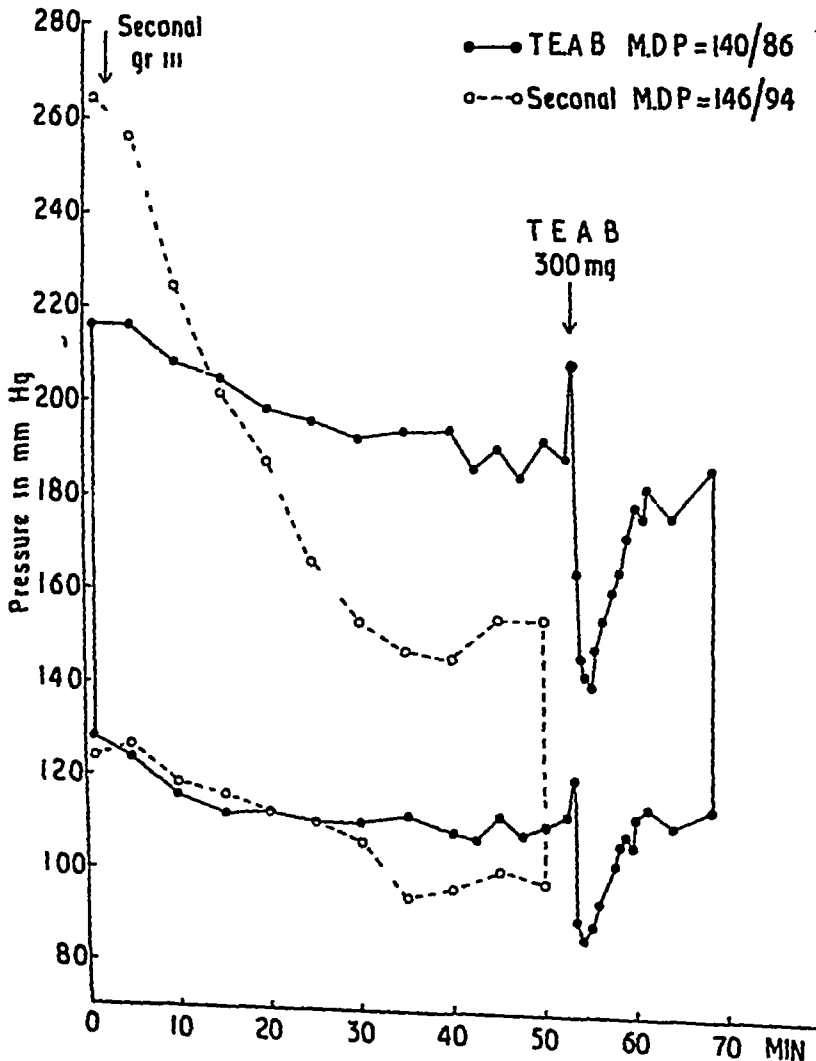
Fig. 7 Case 4 Curves of systolic and diastolic pressures obtained with Seconal and T.E.A.B

Case 5 W B , a housekeeper aged 49, was admitted unconscious on 9th November, 1947 following an overdose of barbiturate For two years she had had asthma, increasingly severe migrainous headaches, tiredness and palpitations with pains in the left groin and back Her blood pressure was 210/130 mm.Hg , her heart was not enlarged, her fundi showed severe changes with early papilloedema, but her renal function tests were normal

26th November, 1947   Initial B P   238/148
                          After T E A B   172/116

2nd December, 1947   Initial B.P   256/154
                          After Seconal 164/113

DISCUSSION

We can conclude that the minimum diastolic pressure obtained with T E A B or with Seconal is the same, when allowance is made for factors which may interfere with a technically satisfactory result It must be emphasised that, with both drugs, the minimum reading may be transitory and, hence, is liable to be missed unless observation is careful In the case of T E A B , apprehension or excitement of the patient, an exaggerated "needle response," and the occasional tachycardia induced by the injection may prevent a true minimum reading, while with Seconal, resistance to the drug, strong visceral impulses (as from a full bladder), and restlessness induced by disturbing dreams, may affect the fall of diastolic pressure

The small difference between the minimum diastolic pressures obtained by the two methods suggests that the component they remove is an entity

T E A B has been shown to block the passage of impulses through the autonomic ganglia (1) It has no action on the vasomotor centre, though, in the experimental animal, it only exerts a depressor effect in the presence of vasomotor tone (1) It has no peripheral action on the sympathetic nerve endings, and, in animals, its action in lowering the blood pressure is prevented by the continuous infusion of adrenalin or angiotonin (1) (14) The fall in blood pressure is not the result of diminished cardiac output (14), and its depressor action in patients with hypertension must be ascribed to interference with efferent vasoconstrictor impulses Neurogenic vasoconstriction is mediated through the vasomotor centre, afferent impulses reaching the centre as a result of external, visceral and psychic stimuli, while the efferent pathways are the sympathetic vasomotor fibres Barbiturates act centrally and are believed to block the reflex arc at a higher level than does T E A B An explanation is thus provided for the similarity of effect of the two drugs We believe that both modify the neurogenic element in hypertension, leaving unaffected increased peripheral resistance produced by other means.

The neurogenic element abolished by T E A B and Seconal would appear to be the same as the " supplemental pressure " described by Alam and Smirk (3), and regarded by them as due to emotional, physical or mental activity It is the element in hypertension responsible for the wide daily variations in blood pressure Gatman, Amin and Smirk (9) suggested that this " supplemental pressure " figured less in renal than in essential

hypertension   This may be true for advanced cases, but, in general, we have found no difference in the reactions of patients with essential hypertension and of those with hypertension secondary to renal disease   While the removal of the neurogenic element may have little effect in the advanced stages of either condition, we have seen earlier cases of renal hypertension show a marked lability of diastolic pressure   The labile neurogenic element is present in both, and may be due either to an increased outflow from the vasomotor centre or to sensitisation of the arterioles to normal vasomotor impulses

The observed fact that, after the removal of this labile element, both T E A B and Seconal leave a similar pressure in patients with hypertension, suggests that this basal pressure has a different significance or mechanism of production   Many factors must be concerned in the maintenance of this underlying basal pressure   Blood pressure is dependent upon the cardiac output, the blood volume, arteriolar tone, the elasticity of the large arteries and the viscosity of the blood   Arterial elasticity affects mainly the systolic pressure and is irrelevant to the present discussion, while the cardiac output and viscosity of the blood have been shown to be normal in hypertension   Changes in the blood volume may affect the blood pressure in normal people and in patients with diminished elasticity of their arterioles, minor changes may produce a more marked effect   The factor mainly concerned in the production and maintenance of the elevated pressure in hypertension seems, however to be the arteriolar calibre ,   and, on this, peripheral resistance largely depends

Three factors are concerned in the maintenance of peripheral resistance or arteriolar tone —

    i   The neurogenic vasoconstrictor reflexes

    ii   The anatomical condition of the arteriolar walls

    iii   An intrinsic tone of the vessels, possibly under the control of humoral factors

If a degree of hypertension persists after removal of the neurogenic element the remaining fixed or basal pressure may result from an increased intrinsic tone of the arteriolar musculature, from rigidity of the vessels consequent upon arteriolar damage, or from a combination of the two

Little is known of the factors affecting the intrinsic tone of the arteriolar bed   though in experimental renal hypertension, humoral mechanisms appear responsible for its maintenance   Provided that the arterioles are still capable of further constriction, comparable neurogenic impulses would seem likely on theoretical grounds, to have greater effect (in terms of increased diastolic pressure), the higher the underlying basal tone   Although a similar neurogenic element has been demonstrated in both essential and

renal hypertension, it would not be legitimate to conclude that the basal component, dependent upon the underlying vascular tone, was the result of the same factors in the two conditions

Whatever the mechanism responsible for the maintenance of a basal diastolic pressure, this pressure is itself liable to variation   In one of our patients, who had manifest uræmia and dehydration, the blood pressure was observed to rise as his condition improved and his weight increased Records of his minimum diastolic pressures, at an interval of a month, showed a considerable change, and emphasise again the similarity of the results obtained by the two methods of measurement —

<div align="center">

9-11 *October*, 1947   Weight 137 lbs   Blood Urea—378 mgms
                            per 100 c c
                            Initial Blood Pressure 170/80 mm Hg
                            After T E A B   136/76
                            After Seconal   122/76

18-19 *November*, 1947   Weight 144 lbs   Blood Urea—204 mgms
                            per 100 c c
                            Initial Blood Pressure 190/100 mm Hg
                            After T E A B   146/94
                            After Seconal   154/92

</div>

In one patient with acute glomerular nephritis, whose blood pressure was 164/98 mm Hg , neither T E A B nor Seconal produced a fall in the diastolic pressure, although the casual diastolic pressure was only 60 mm Hg on recovery from the acute attack   A similar observation has been made with T E A B in another case, suggesting that the neurogenic element plays little part in the hypertension of acute nephritis, which appears to represent a simple increase of the basal level

Experimental evidence (20) suggests that a labile hypertension may, in time, lead to organic change in the arteriolar walls, starting a " vicious circle " which is regarded as an important factor in the genesis of the malignant phase of both essential and renal hypertension   In Fig 3, patients who had papillœdema at the time their basal pressure was measured are indicated by a different symbol from the remainder   These patients with the malignant type of hypertension, whether essential or renal in origin, show a high minimum diastolic pressure, measured by both methods Pickering (17) has shown that there is a correlation between the height of the diastolic pressure and the occurrence of raised intracranial tension and papillœdema   The finding of a high minimum diastolic pressure in a patient with hypertension seems to indicate that the disease is approaching the malignant phase, though both the duration of the hypertension and the rapidity of its development must also be of importance   The higher the minimum diastolic pressure, the more likely is there to be gross organic arteriolar damage

Levinson, Reiser and Ferris (13), who employed repeated tests with Tetra-ethyl-ammonium Chloride, have suggested that fluctuating neurogenic and humoral mechanisms interact as factors in clinical hypertension We would suggest that various factors, such as organic structural change in the arterioles, intrinsic vascular tone of humoral or other origin and the circulating blood volume, interact to produce the basal blood pressure, while the casual blood pressure results from superadded neurogenic tone We believe that the minimum diastolic pressure, observed after the administration of Seconal, Amytal or T E A B , is the best indication, at present available, of this basal pressure

SUMMARY

1 The minimum diastolic pressures obtained with Tetra-ethyl-ammonium Bromide and with a rapidly acting Barbiturate (Seconal) in cases of hypertension are practically identical

2 It is suggested that both drugs act upon the neurogenic element of hypertension, though at different levels of the reflex arc

3 Patients in the malignant phase of hypertension show a high minimum diastolic pressure

4 No difference has been detected in the response of patients with Essential Hypertension and those with hypertension secondary to chronic renal disease to these drugs, but the neurogenic element appears to be of little significance in the hypertension of acute glomerular nephritis

5 The significance of the basal blood pressure is discussed

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A SIMPLE METHOD OF ESTIMATING CLOT RETRACTION WITH A SURVEY OF NORMAL VALUES AND THE CHANGES THAT OCCUR WITH MENSTRUATION

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A VARIETY of techniques have been suggested for the quantitative measurement of clot retraction (2, 3, 4, 9, 13, 14, 16, 18)   They all depend essentially upon the measurement of the volume of serum expressed by the retracting clot   The methods range from the extremely simple one of Fonio (10) who measured the height of the column of serum above the clot, a method which takes no account of the lateral retraction of the clot, to the complex technique of van Allen (3) in which expressed serum is collected by centrifuging the clot against a perforated disc, a method which the author himself admits is of no value when dealing with poorly retracted clots Of the various methods described, that of Macfarlane (14) is probably the most satisfactory   In this method 5 ml of blood are placed in a graduated centrifuge tube which is then closed with a cork through which has been passed a glass rod with a button expansion near its lower end, so arranged that the expansion lies well below the surface of the blood   The clot contracts on to the glass rod and when retraction is complete the clot is removed by raising the rod   The volume of expressed serum and red cells is then read directly from the graduated scale   This method is generally satisfactory when clot retraction is normal   It has, however, certain drawbacks Firstly, for accurate work it is desirable to make at least three estimations at a time This necessitates the use of a rather larger volume of blood than may be easily obtained especially in children   Secondly, if the clot adheres at any point to the wall of the tube it is extremely difficult to remove the cork and separate the clot from the tube, without moving the glass rod and so tearing the clot   This is particularly true with poorly retracted specimens Thirdly the glass rod tends to cut out of such clots as they are being removed Finally when clot retraction is extremely deficient, and these are the cases where accurate measurement is of the greatest interest, the clot may retract so little that although not adherent to the wall of the tube, it may be almost completely in contact with it   On attempting to remove such a clot a

partial vacuum is created between the lower end of the clot and the bottom of the tube   This tends to suck fluid out of the clot and will often cause it to break

In the course of an investigation on purpura due to drug hypersensitivity it became necessary to measure relatively minor differences in clot retraction using small quantities of blood   To overcome some of the difficulties outlined, the following method was devised

Method

The apparatus, shown in Fig 1, consists of a piece of glass tubing down the whole length of the outside of which a strip about 0 5 cm wide has been ground with a carborundum block   The tube has a uniform bore of 8 mm and is about 12 cm long   The necessity for using standard conditions in the
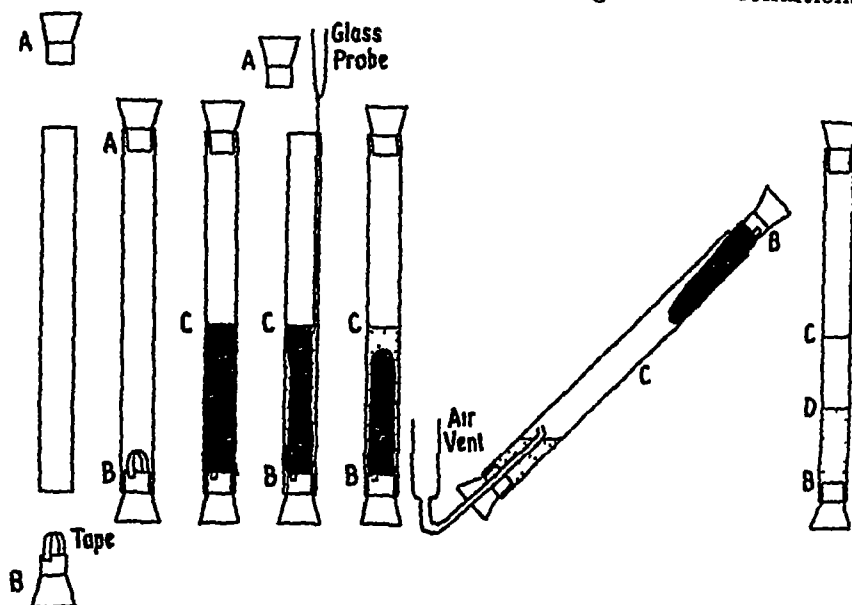
Fig 1   Diagram of apparatus used in estimating clot retraction   With well retracted clots it is not necessary to use the air vent

estimation of clot retraction has frequently been emphasised (8, 18) and tubes of these dimensions have been used throughout this investigation The tube is closed at each end with a No 2 bark cork   The corks (A and B in figure) should be well made and have their narrower ends cut flat and at right angles to the long axis of the cork   A small loop of tape is placed in the lower end of the tube with its ends anchored between the wall of the tube and the cork B   The ends of the loop of tape should not extend outside the tube as under such circumstances they form a wick along which blood will escape

Clot retraction is measured as follows   The cork A is removed and 2 ml of blood are placed in the tube   The tube is then recorked   When clotting has occurred the tube is placed in an incubator at 37°C and examined

at the end of 1 hour   In most cases retraction will have occurred without adhesion to the wall of the tube   If the clot is adherent at any point the adhesion may be broken down with a fine glass probe which can be introduced through the upper end of the tube without disturbing the clot The tube is then incubated for a further period of three hours after which it is left on the bench overnight   The retraction is read on the following morning   To do this, the level of the serum (C) is marked on the ground glass strip with a sharp pencil   The distance of the mark C from the top of the cork B represents the height of the original column of blood   The tube is now inverted so that the serum drains away from the clot which is removed by extracting the cork B with its attached piece of tape   The tape has become incorporated in the clot and even if retraction is extremely poor the clot will not break as it is removed   The clot is discarded   It will be observed that the narrower end of the cork B which has been in the tube has been compressed and that there is a well defined ridge surrounding the cork, corresponding to the rim of the tube   The cork is now replaced so that the rim of the tube comes into contact with this ridge , the cork then occupies exactly the same position it occupied before   That this can be done accurately was shown when the lower corks were removed from a series of such tubes containing fluid to a marked level   No change in the fluid level was observed when the corks were replaced and the tubes again stood upright   Finally the tube is placed in its original position with the cork A uppermost and the level (D) of the serum is marked on the ground glass strip   The distance of the mark D from the top of the cork B represents the height of the column of expressed serum   The clot retraction is calculated by expressing the volume of serum as a percentage of the original volume of blood   Thus since the tubes are of uniform bore,

$$\text{retraction} = \frac{\text{height of column of serum}}{\text{height of column of blood}} \times 100 = \frac{BD}{BC} \times 100\%$$

If these precautions are taken, any serious degree of adhesion of the clot
to the wall of the tube will be uncommon   It is important to note however
that if there is much adhesion the results will be unreliable even if the clot
is freed at the end of the first hour's incubation   Since this investigation
was completed it has been found simpler and equally satisfactory to coat
the inside of the tubes with a thin layer of paraffin wax

Error of method

Thirty-six determinations of clot retraction were made consecutively
on blood obtained from the same patient over a period of three minutes
The distribution of the thirty-six observations is shown in Fig 2   The
mean retraction was 52 1%   The readings ranged from 50%—55%   The
standard deviation was 1 4 and the coefficient of variation 2 7%
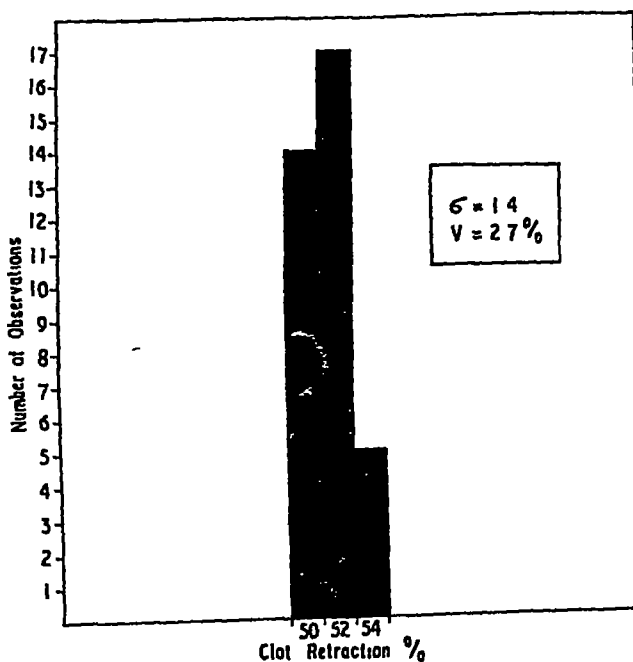
Fig 2   Distribution of the 36 estimations of clot retraction on blood taken from the same patient

Changes in retraction depending on variations in the packed cell volume

Since clot retraction varies inversely with the packed cell volume
(5, 13, 14, 15, 19) it follows that a low haematocrit reading will tend to mask
a reduction in clot retraction   However, when clot retraction is greatly
reduced it becomes independent of the packed cell volume and clots formed
from plasma alone may then show no retraction   As the study of clot
retraction is of most interest in the haemorrhagic diseases it is clearly desirable
to have some method of correcting the clot retraction reading for variations
in the haematocrit.   Various procedures have been proposed (2, 5, 14, 15)

but as these gave widely different results with the method of estimating clot retraction just described it was decided to investigate the changes in clot retraction that occur with alterations in the packed cell volume This was done in the two following ways

1 *By diluting whole blood with plasma without the use of anticoagulants* Blood was withdrawn from a normal individual using a 10 ml record syringe with a two-way tap and measured quantities were placed in waxed tubes Some of these tubes were then centrifuged slowly to separate off the red cells whilst leaving the platelets in the supernatant plasma This plasma was transferred in measured quantities in a waxed pipette to the blood in the remaining tubes The blood and plasma were quickly mixed and 2 ml of each mixture placed in a clot retraction tube The remainder of each mixture was then heparinised and its haematocrit determined Clot retraction was thus estimated at 10 or 11 different haematocrit levels on the same patient Seven healthy individuals with normal platelet counts were investigated in this way (Table I) In each case it was found that the relationship between the haematocrit and clot retraction was a straight line These lines are shown superimposed in Fig 3 which has been constructed by plotting all
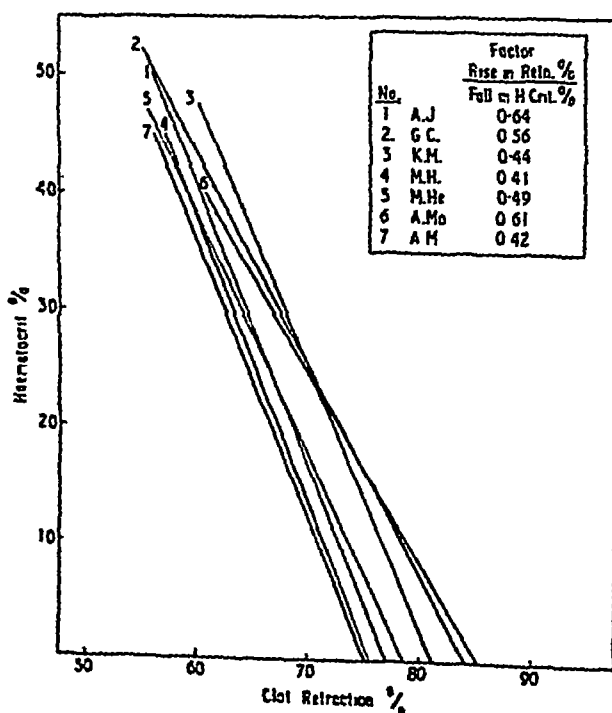
TABLE I

Effect on clot retraction of diluting whole blood with homologous plasma using no anticoagulants

Sex	Haematocrit % (RBC+WBC)	Clot retraction %	Serum haematocrit % (RBC+WBC)	ECCV %*	Sex	Haematocrit % (RBC+WBC)	Clot retraction %	Serum haematocrit % (RBC+WBC)	ECCV %*
A M F (M H)	44	57	15.5	7.8	**A M** M (M H)	45	57	13	5.4
	40	57	11.5	9.6		39	59	13	9.7
	35.5	59	8.5	10.5		38	59	10.5	9.2
	31	64	11.0	12.0		33.5	62	6	8.2
	27.5	62	6.5	14.3		28.5	65	3	8.5
	22	64	3.5	16.2		22.5	62	0.5	15.8
	18	65	4.5	19.0		10	65	1.5	17
	14.5	67	1.0	10.2		15	65	2.5	21.6
	8.5	70	0.5	21.9		8	77	0.25	15.2
	2.5	65	0	32.5		3.5	71	0.25	25.7
	0	76	0	24		0	79	0	21
A No F (G C)	39.5	62	15	7.8	**A No** M (G C)	51	53	22	7.7
	36	63	10.5	7.6		40.5	66	24	9.3
	35.5	62	10	8.7		35.5	64	13.5	9.1
	32.5	07	8	5.9		30	68	10.5	9.1
	26.5	08	3.5	7.0		25	08	3.5	8.7
	20.5	72	2	8.0		21.5	00	1.5	10.5
	10	72	1	10.4		10.5	-7	1.5	8.0
	11	70		10.8		11.5	78		2

* 1 C.C.V. = Extra corpuscular volume of the clot

the points given in Table I and drawing the best line between them for each case investigated   To preserve clarity, however, the individual points are not shown in the figure   The factor $\dfrac{\text{Rise in clot retraction}\%}{\text{Fall in hæmatocrit}\%}$ was calculated for each case   The ratio ranged from 0 41—0 64   The value 0 5 was taken as a mean which gave the following formula for calculating the retraction at any given hæmatocrit level—

$$\text{Retraction} = R - \frac{(H_2 - H_1)}{2}$$

where R = Observed retraction %

H_1 = Observed hæmatocrit %

H_2 = Hæmatocrit % at which the clot retraction is required to be known

2   *By observing clot retraction in patients suffering from severe post-hæmorrhagic and non deficient anæmias*   Retraction was estimated before and after treatment   The results of this investigation are shown in Table II   In each case the clot retraction on recovery was predicted on the basis of the above formula   It will be seen that the agreement between the predicted retraction and the retraction observed on recovery is very close, thus justifying the use of the formula

Method of stating results

Of the various methods suggested for expressing clot retraction quantitatively (2, 3, 4, 10, 14, 18) that of Macfarlane (14) in which the volume of serum and red cells extruded from the clot is expressed as a percentage of the original volume of blood, seems to be the simplest and most satisfactory   Aggeler and his colleagues (1, 2) have suggested that retraction should be expressed in terms of the extracorpuscular volume of the clot which they calculate as the difference between the volume of the clot and the volume of the packed cells contained in the blood before clotting occurred   This calculation ignores the volume of red cells extruded with the serum, a volume which in normal people may vary from 4%—26 5% of the total volume of extruded serum and red cells (see Table III)   It is clear that the volume of extruded red cells must be taken into account in calculating the extracorpuscular volume of the clot   The only author to have realised the importance of this appears to be van Allen (3) who deducted the volume of red cells in the serum from the volume of serum plus red cells extruded from the clot and calculated this as a percentage of the original volume of plasma   Calculation of the difference between the volume of serum and the volume of plasma in this way gives a method of estimating the true extracorpuscular volume of the clot which can be expressed as a percentage of the original volume of blood   It was thought at first that this might be a satisfactory method of expressing clot retraction and that the extracorpuscular clot volume

TABLE II

Table comparing the clot retraction in anaemic patients after recovery with the retraction predicted from the haematocrit (RBC + WBC) and retraction readings obtained during the anaemic stage

Patient	Diagnosis	y	Original haemato crit % (RBC + WBC)	Final haemato crit % (RBC + WBC)	Original retraction %	Predicted final retraction %	Actual final retraction %	Original serum haemato crit % (RBC + WBC)	Final serum haemato crit % (RBC + WBC)	Original ICCV %†	Final ICCV %†
A B	PHA	1	20	17	71	66	67	+	18	00	81
J C	PHA	4	20	13.5	65	63	57	1	21.5	157	118
A G	PHA	1	20.5	12	60	63	63	1	10	121	107
M I	IDA	1	31.5	13	66	62	60	14	17	87	80
J B	PHA	4	17.5	10	70	68	66	+	21.5	87	82
D I	PHA	M	18	11.5	90	68	65	7	17.5	07	19
A H	IDA	M	12	10	74	61	60	0.25	8	112	97

L.* PHA = Post haemorrhagic anaemia

IDA = Iron deficient anaemia

† ICCV = Intracorpuscular clot volume

TABLE III

Clot retraction in normal individuals

Men						Women						
No	No of experiments	Blood hæmatocrit % (RBC+WBC)	Retraction %	Serum hæmatocrit % (RBC+WBC)	Corrected retraction %	No	Week of menstrual cycle	No of experiments	Blood hæmatocrit % (RBC+WBC)	Retraction %	Serum hæmatocrit % (RBC+WBC)	Corrected retraction %
1	4	48	55		57	34	4	4	39	55	6 5	52
2	4	44	59		59	35	1	4	40 5	55	0	53
3	7	49 5	60		63	36	1	4	40 5	51	4	49
4	4	40	54		56	37	1	4	42	51	8	40
5	4	40 5	60		61	38	2	4	40 5	60	10	58
6	4	48 5	56		58	39	4	4	46	52	13	53
7	3	44 5	54		54	40	3	4	37	62	8 5	58
8	4	47	56		57	41	4	4	44	51	0	51
9	4	48	51		53	42	3	3	40	60	15	58
10	4	44	56		56	43	1	5	43	54	9 5	53
11	4	44 5	58		58	44	2	5	43 5	50	14	55
12	4	50	54		57	45	1	4	42 5	57	15 5	50
13	4	45 5	53		53	46	2	5	48	52	13	54
14	3	51	54		57	47	4	5	46 5	55	16	50
15	4	44	50		50	48	4	5	44	55	10 5	55

percentage would not vary with alterations in the packed cell volume   It
will be seen, however, from Tables I and II in which the extracorpuscular
clot volume percentages (E C C V %) have been calculated for various
hæmatocrit levels, that except in one case of post-hæmorrhagic anæmia
(J B , Table II), the extracorpuscular clot volume percentage has varied
inversely with the hæmatocrit reading   The estimation of the extra
corpuscular clot volume therefore, shows no advantages over the simpler
calculation of clot retraction in which the volume of extruded serum and red
cells is expressed as a percentage of the original volume of blood   This
method has, therefore, been used throughout this investigation   For
reasons given below it was decided to adjust all the results to a standard
hæmatocrit reading of 45%

Normal range of clot retraction

Clot retraction was estimated in 33 healthy men and 33 healthy women
whose ages ranged from 18 to 62 years   The majority of those investigated
were medical students and nurses and were under 30 years of age   In every
case the hæmatocrit (R B C + W B C) was estimated and the platelets
shown to be numerous by the method described by Giam (12)   In most
cases the volume of cells extruded in the serum was estimated after
centrifuging, as in the determination of the blood hæmatocrit   The volume
of the packed cells was expressed as a percentage of the total volume of
serum and cells, this figure being referred to as the serum hæmatocrit   The
results are shown in Table III and the frequency of distribution of the
clot retractions is shown in Fig IV   The mean retraction was 55 18%
the range being from 50%—62%   A further analysis of these figures is shown
in Table IV   Briefly, they show that there was no statistically significant
difference between the mean clot retraction in the men and the women
There was likewise no significant difference between the mean clot retraction
of the women who were in the first week of the menstrual cycle and those in
the remaining period of the cycle   The packed cell volumes of the individuals
investigated, however, varied considerably, the lowest being 37% and the
highest 51%   As clot retraction varies inversely with the hæmatocrit, all
the readings were corrected to a standard hæmatocrit of 45% by means
of the formula given above   That this is justifiable for clot retractions
within the normal physiological range of hæmatocrit readings is shown by
calculating the coefficient of correlation between the blood hæmatocrit and
the clot retraction   This shows a statistically significant degree of negative
correlation (r = -0 3162, P = 0 01 — 0 02) showing therefore that even at
physiological levels clot retraction varies with the blood hæmatocrit

Analysis of the clot retraction figures when these have been adjusted
to a standard hæmatocrit of 45% shows that the difference between the
mean retractions for the men and women has now risen from 1 05% to 1 3%
This difference is due almost entirely to the figures obtained for women in
the first week of the menstrual cycle, the mean for this group being 3 51%

TABLE IV

Analysis of clot retraction in normal individuals

	N (No of individuals)	Mean clot retraction %	Difference between means	t	P	Mean clot retraction % adjusted to haematocrit of 45%	Difference between means	t	P
Men	33	54.07				55.94			
Women	31	55.72	1.05	1.486	0.1–0.2	54.64	1.3	1.86	0.005
Women in first week of menstrual cycle	7	53.86				52.43			
Women in remaining period of cycle	24	56.28	2.42	1.70	0.085	55.16	3.03	3.042	0.01–0.001
Men	33					55.94	0.48	0.078	0.5

lower than the mean for the men, whereas the mean clot retractions in the second, third and fourth weeks of the menstrual cycle differ in no case from the mean for the men by more than 0 69%   The difference between the means of the clot retractions of the women who were in the first week of the menstrual cycle and the rest of the women is 3 03% which is statistically highly significant (t = 3 04, P = 0 01 — 0 001)   In other words, the clot retraction of the women who were in the first week of the menstrual cycle is significantly lower than that of those in the remaining period of the cycle That there is no statistically significant difference between the retraction in the men and in the women who were not menstruating can readily be seen by comparing the retractions of the two groups   The means are practically identical, the difference being only 0 48%, a difference which is certainly not
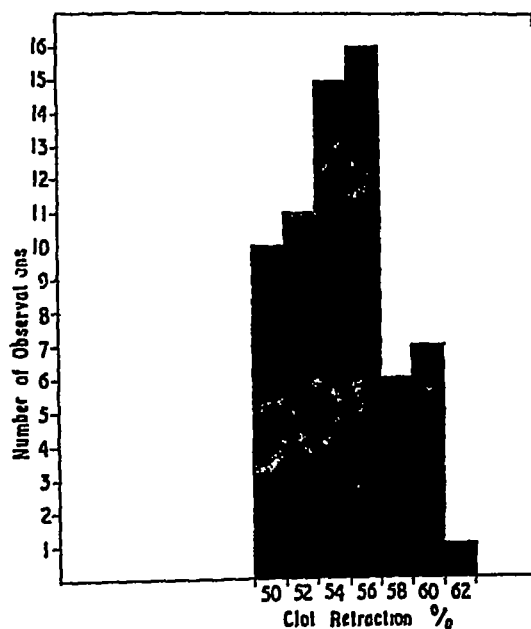
Fig 4   Distribution of clot retraction (uncorrected) in 33 healthy men and 33 healthy women

significant (P = 0 5)   The frequency of distribution of the clot retractions of the men and women after the figures have been adjusted to a standard hæmatocrit of 45% is shown in Fig 5   The positions on the histogram of the results obtained in the women who were in the first week of the menstrual cycle are indicated in grey   The distribution of the figures obtained in the men and in the women in the remaining period of the cycle is shown in black   It is clear from the foregoing that the women in the first week of the menstrual cycle constitute a different population and these have been excluded in assessing the normal range   The mean clot retraction of the men and of the women who were not in the first week of the menstrual cycle is 55 75% The standard deviation is 2 45 and the coefficient of variation 4 4%   If the range is calculated as M± 3σ, clot retraction may be expected to vary from 48 4% to 63 1%

The reason for this reduction in clot retraction in the week following the onset of menstruation is not clear   It has been shown that there is a positive correlation between the platelet count and clot retraction (13, 19) and it was thought at first that these changes in clot retraction might be due to the cyclical changes in the platelet count that are known to occur in menstruating women   Genell's (11) report that the platelet count falls suddenly with the onset of menstruation and then rises slowly to reach the premenstrual level in about five days seemed to support this view as these changes would correspond fairly exactly with the changes observed in clot retraction   There is, however, no agreement as to the phase of the menstrual cycle in which the platelet count is lowered and Dameshek (6) and Pohle (17)
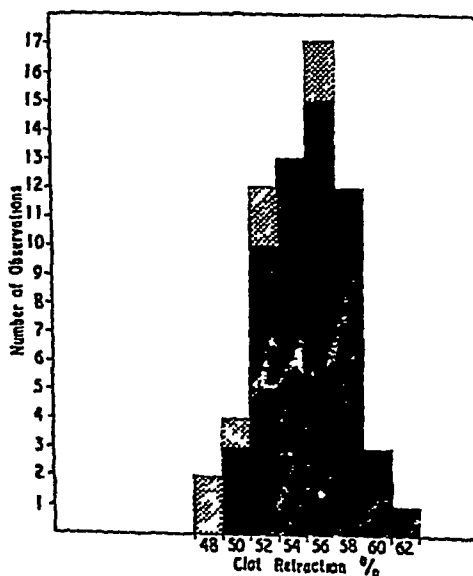
Fig 6   Distribution of clot retraction (adjusted to a hæmatocrit of 45%) in 33 healthy men and 31 healthy women   The stippled areas represent the position on the histogram of the results in the seven women in the first week of the menstrual cycle

have both found that the fall in the platelet count usually occurs in the premenstrual period and that the count rises rapidly with the onset of menstruation   It is clear therefore that too little is known about the changes in the platelet count that occur in menstruation to permit conclusions being drawn as to a possible relationship of such changes to the reduction in clot retraction which has been observed in the first week of the menstrual cycle   It must therefore be stated that the cause of this reduction in clot retraction is not known

One further point comes out in an analysis of these figures   It has been claimed (7) that the extravasation of a large number of red cells from the clot is indicative of a hæmorrhagic tendency   van Allen (3) on the other hand considered the number of extravasated cells to be proportional to the

rate of clot retraction   This latter view is certainly not entirely correct because, as can be seen from Table V, the number of extravasated red cells can be greatly reduced if clot retraction is accelerated by the use of

TABLE V

Effect of the addition of thrombin to blood on the volume of red cells extruded in the serum

Patient	2 ml blood + 0 1 ml saline		2 ml blood + 5 units thrombin in 0 1 ml saline	
	No of experiments	Serum hæmatocrit %	No of experiments	Serum hæmatocrit %
T D	2	5	4	1 5
N O	3	3	4	1
W A	4	9	4	1
B L	4	10	4	1
M I	4	8	4	1
J A	4	12	4	2

thrombin   It is clear from the figures given in Tables I and II that the volume of red cells in the serum varies directly with the blood hæmatocrit That this relationship holds good at normal hæmatocrit levels can be shown by calculating the coefficient of correlation between the blood and serum hæmatocrit from the figures given in Table III   This shows a highly significant correlation (r = 0 644, P = 0 001)   It would, therefore, seem clear that whatever other factors are involved the blood hæmatocrit must be taken into account in assessing the significance of the volume of red cells in the serum

SUMMARY

1   A simple method of estimating clot retraction is described   Clot retraction is determined by calculating the volume of serum and red cells extruded from the clot as a percentage of the original volume of blood All clot retraction results are adjusted to a standard hæmatocrit level of 45% by the application of a formula

2   Thirty-six determinations of clot retraction on the same patient over a short interval show that with this method $\sigma = 1 4$, $V = 2 7\%$

3   Clot retraction in women in the first week of the menstrual cycle is significantly lower than in women in the remaining period of the cycle This difference is only apparent if the figures are corrected for variations in the blood hæmatocrit   There is no significant difference between the corrected clot retraction figures obtained for men and for the women who were not menstruating

4 In calculating the normal range of clot retraction it is necessary to eliminate the women in the first week of the menstrual cycle Taking the range as $M \pm 3\sigma$, the corrected clot retraction figures in men and in women not in the first week of the menstrual cycle vary from 48 4%—63 1%

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THE PATHOGENESIS OF
THROMBOCYTOPENIC PURPURA DUE TO HYPERSENSITIVITY TO SEDORMID

(ALLYL-ISOPROPYL-ACETYL-CARBAMIDE)

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INTRODUCTION

Thrombocytopenic purpura due to hypersensitivity to foods and drugs, though uncommon has been reported in association with a very large number of different substances, particularly sedormid (15, 23, 41, 53, 54, etc), the arsenobenzol compounds (4, 21, 22, 40, 42, 70, 86, etc), and the sulphonamides (16, 29, 35, 38, 65, 68, etc)   Other substances which have occasionally been recorded as causing thrombocytopenic purpura are quinine (5, 66), quinidine (11), gold (33, 45), bismuth (8), iodine compounds (14, 15), chrysarobin (55)   benzol (71), phenobarbitone (2, 9), alurate (allyl-isopropyl barbituric acid) (86), nirvanol (37), sodium salicylate (64), insulin (76)   and   Leg stocking colour " preparations (44)   Newcomb and Deane (56) have described a case of thrombocytopenic purpura with granulopenia which followed treatment with thiourea and Imerman and Imerman (36) have reported a similar case due to dinitrophenol Thrombocytopenic purpura has also been recorded as a manifestation of hypersensitivity to insect bites (25) and to pertussis vaccine (39)   Hertzog and Roscher (34) have reported two cases of thrombocytopenic purpura due to the intravenous administration of colloidal silver   The association was however not very definite as both patients were also receiving arsenobenzol compounds   Finally several authors (19, 73, 80, 84) have claimed that thrombocytopenic purpura may on occasion be due to hypersensitivity to various foodstuffs   That a given food or drug has caused an attack of purpura can only be considered to be proved if the administration of a test dose of the substance after recovery from the original attack of purpura

produces a further hæmorrhagic episode In several of these reports no test dose was given and however good the circumstantial evidence, such cases must be regarded as unproved

The drug which has been most commonly associated with thrombocyto penic purpura is the hypnotic sedormid (41), the active principle of which is allyl-isopropyl-acetyl-carbamide It is one of the group of open chain ureides, other commonly used members of this group being adalin and bromural The structural formulæ of these drugs are shown in Fig 1

Sedormid

Allyl-isopropyl-acetyl-carbamide

$$NH - CO - CH \begin{array}{c} CH(CH_3)_2 \\ \\ CH_2\ CH\ CH_2 \end{array}$$

$$C = O$$

$$NH_2$$

Adalin

Diethyl-bromo-acetyl-carbamide

$$NH - CO - C \begin{array}{c} C_2\ H_5 \\ -Br \\ C_2\ H_5 \end{array}$$

$$C = O$$

$$NH_2$$

Bromural

Isopropyl-bromo-acetyl-carbamide

$$NH - CO - CH \begin{array}{c} CH(CH_3)_2 \\ \\ Br \end{array}$$

$$C = O$$

$$NH_2$$

Fig 1. Structural formulæ of three commonly used open chain ureide hypnotics

In spite of the striking chemical resemblance of sedormid to the other members of this group of hypnotics, no other open chain ureide appears ever to have caused purpura The possibility that a patient who had recovered from an attack of purpura due to sedormid might also be hypersensitive to other, related compounds was investigated by Hadorn (32) He gave such a patient at different times abasin, adalin, bromural and phanodorm, the first three being open chain ureides and the last a barbiturate None of these drugs caused any fall in the platelet count or any hemorrhagic manifestations although a few days earlier the patient had developed a severe attack of thrombocytopenic purpura after being given a test dose of sedormid

produces a further hæmorrhagic episode   In several of these reports no test dose was given and however good the circumstantial evidence, such cases must be regarded as unproved

The drug which has been most commonly associated with thrombocyto-penic purpura is the hypnotic sedormid (41), the active principle of which is allyl-isopropyl-acetyl-carbamide   It is one of the group of open chain ureides, other commonly used members of this group being adalin and bromural   The structural formulæ of these drugs are shown in Fig 1

Sedormid

Allyl-isopropyl-acetyl-carbamide

$$
\begin{array}{l}
NH - CO - CH \diagup^{CH(CH_3)_2} \\[2pt]
C = O \qquad\qquad \diagdown CH_2\ CH\ CH_2 \\[2pt]
NH_2
\end{array}
$$

Adalin

Diethyl-bromo-acetyl-carbamide

$$
\begin{array}{l}
NH - CO - C \diagup^{C_2 H_5}_{\diagdown C_2 H_5} {-}Br \\[2pt]
C = O \\[2pt]
NH_2
\end{array}
$$

Bromural

Isopropyl-bromo-acetyl-carbamide

$$
\begin{array}{l}
NH - CO - CH \diagup^{CH(CH_3)_2} \\[2pt]
C = O \qquad\qquad \diagdown Br \\[2pt]
NH_2
\end{array}
$$

Fig 1   Structural formulæ of three commonly used open chain ureide hypnotics

In spite of the striking chemical resemblance of sedormid to the other members of this group of hypnotics, no other open chain ureide appears ever to have caused purpura The possibility that a patient who had recovered from an attack of purpura due to sedormid might also be hypersensitive to other, related compounds was investigated by Hadorn (32) He gave such a patient at different times abasin, adalin, bromural and phanodorm, the first three being open chain ureides and the last a barbiturate None of these drugs caused any fall in the platelet count or any hæmorrhagic manifestations although a few days earlier the patient had developed a severe attack of thrombocytopenic purpura after being given a test dose of sedormid

who developed thrombocytopenic purpura during treatment with sul-
phathiazole   No purpura developed and there was no fall in the platelet
count when this patient was given a second course of the drug a fortnight
later   It is however possible that the purpura in this case was due to the
infection for which the patient was being treated rather than to the
sulphathiazole   In many of the reported cases of sedormid purpura the
relationship of the drug to the attack of purpura has been established beyond
doubt by the administration of test doses of the drug after the patient has
recovered from the original attack of purpura (23, 43, 46, 50, 54, etc)
The platelet count has usually been reported to have fallen to a very low
figure within a few hours and in many cases spontaneous purpura has also
resulted   Similar results have been reported following the administration
of test doses of the appropriate substance to patients who have recovered
from purpura due to other drugs (5, 21, 22, 70, etc)

The bone marrow findings in purpura due to sedormid have varied
considerably   Hadorn (32) and Lieberherr (43) considered that the
megakaryocytes were reduced in number   Hadorn thought they were
morphologically damaged and Lieberherr reported that many of the
megakaryocytes contained vacuoles   Moeschlin (54) however, in ten cases,
found only slight morphological changes in the megakaryocytes which he
considered to indicate an inhibition of maturation   Leitner (41) has reported
similar findings   Buchler (12) and Falconer and Schumacher (23) on the
other hand have reported normal findings in the bone marrow   A similarly
wide range of findings has been reported in cases of thrombocytopenic
purpura due to other drugs   According to Leitner (41) the bone marrow
in purpura due to drug hypersensitivity most commonly shows a normal or
only slightly decreased number of megakaryocytes, which show no
morphological changes   Less commonly they may show some inhibition of
maturation   These changes in the bone marrow have been held by many
workers to account for the thrombocytopenia   Thus, in purpura due to
sedormid the thrombocytopenia has been attributed to damage to
megakaryocytes (32) or to a functional inhibition of platelet formation
(43, 50)   Lieberherr (43) considered that although the functional disturbance
of the megakaryocytes was in part responsible for the fall in the platelet
count, it was probable that there was also some other factor involved   He
suggested that this factor probably acted on the platelets in the peripheral
blood   Moeschlin (54) also thought that the changes in the marrow were
insufficient to account for the thrombocytopenia, as he was able to show
that the fall in the platelet count could occur in as short a time as 30-60
minutes after giving a test dose of sedormid to a sensitive patient   A
similar time relationship had previously been reported by Falconer and
Epstein (21) in cases of purpura due to arsenobenzol compounds   The very
rapid fall in the platelet count after the administration of sedormid suggested
to Moeschlin that the platelets might be destroyed in the peripheral blood.

He therefore tried to demonstrate a circulating thrombocytolysin by transfusing a woman with a normal platelet count with blood from a case of thrombocytopenic purpura due to sedormid   In the first experiment 200 ml of blood with a platelet count of 74,000 per c mm was used   In the second experiment 500 ml of blood from the same donor, with a platelet count of 4,000 per c mm was transfused into the same recipient   On neither occasion did the platelet count in the recipient's blood change significantly nor did she develop purpura   Moeschlin therefore concluded that there was no circulating thrombocytolysin in the blood and that the platelets were damaged by some unknown mechanism   Another hypothesis which might explain the rapid fall in the platelet count has been suggested by Quick Ota and Baronofsky (63), who showed that if anaphylactic or peptone shock was induced in various laboratory animals the platelet count fell to very low levels within a few minutes   If the blood was collected just before the fall occurred the platelets were found to be adhering to each other in clumps   They considered the thrombocytopenia was due to the agglutinated platelets becoming lodged in the capillary bed   They suggested that a similar mechanism might be the cause of the low platelet counts found in thrombocytopenic purpura, a suggestion which had previously been made by Leischhacker and Walterskirchen (26), Lieberherr (43) and Falconer and Epstein (21)   The latter authors cited two cases of thrombocytopenic purpura, one due to bismarsan and one to neoarsphenamine, in each of which the platelet count rose following the subcutaneous injection of adrenaline, an observation which Falta (24) had previously made in a case of purpura due to sedormid   Falconer and Epstein suggested that the adrenaline ' increased the circulatory tone by contracting the capillary bed, and restored to the general circulation platelets that were temporarily out of circulation "   It would though seem equally likely that the rise in the platelet count was due to the action of adrenaline in causing contraction of the spleen and consequently expressing into the general circulation platelets previously shut away in the splenic sinusoids and consequently not exposed to the action of the drug

effect of the drug on the platelets, as the platelet count fell only slightly as a result of patch testing   It should howevei be noted that on no occasion did the capillary resistance fall below normal limits even in the test areas

A few attempts have been made to transfer this type of drug hypersensitivity passively to normal subjects   Loewy (46) reported negative results in one case of sedormid purpuia   He did not state what method he used   Moeschlin (54) attempted unsuccessfully to transfer hypersensitivity to sedormid by transfusing whole blood from a case of sedormid purpura   No significant alteration in the platelet count or other sign of hypersensitivity was observed when the iecipient was subsequently given sedormid by mouth   Positive iesults to passive transfer tests have been reported in a case of thiombocytopenic purpuia due to quinine (5), and in one due to quinine and ergot (59)   In each case the iecipient developed a cutaneous wheal when given quinine   These two reports lack detail and it is difficult to know how much weight to attach to them especially as the lesion pioduced was urticarial and urticaria is not a characteiistic of this type of purpuia   The same comment also applies to the above mentioned claim by Peshkin and Millei (59) that scratch testing with quinine in a case of purpura due to quinine and eigot produced cutaneous wheals Jones and Jacobs (37) have attempted to tiansfei this type of hypersensitivity to a guinea pig   They injected the animal intraperitoneally with serum from a case of purpura due to nirvanol and subsequently suspended the animal's uterus in a bath to which nirvanol was added   The nirvanol produced no increase in the uterine contractions

It appears therefore that this type of purpura is a manifestation of drug hypersensitivity   It has frequently been demonstrated that once the hypersensitivity is established purpura develops within a short time whenever the appropiiate drug is taken but attempts to elucidate the mechanism by which the purpuia is produced have been, in general, unsuccessful

Three of the characteiistic findings common to all types of thrombocytopenic puipura are the low platelet count, the reduced clot retraction and the raised capillary fragility, associated with the appearance of hæmorrhages in the skin and elsewhere   These three phenomena, as they occur in sedormid purpuia, are analysed in the present paper   It is shown that the platelets are agglutinated by sedoimid and that sedormid reduces clot retraction in vitro in the blood of patients who have recovered from sedormid purpura   Finally, it is shown that sedormid has a distinct and separate effect on the skin capillaiies, its application to the skin producing local hæmorihages without causing any significant change in the platelet count

Case Reports

Case 1   N H , female, born 1901   In 1942 this patient began to take sedormid at her menstrual periods   She took 28 or 29 tablets in the course of a year   In July, 1943, on the second day of a menstrual period in which she

bled excessively, she noticed severe bruises on the thighs and small red spots all over her body   There was also bleeding from the gums   This hæmorrhagic episode cleared up after about a week   She does not remember if she took sedormid for this particular menstrual period

In the following month (August, 1943), she again noticed bruises and small red spots on her body   These appeared two or three days before her menstrual period began   She is certain that she took no sedormid between this attack of purpura and the previous one in July, 1943

Following this attack she took no more sedormid until the 5th February, 1945, when she took 1½ tablets (0 375 grams)   Two days later her menstrual period began at the expected time   On the evening of the second day of this period she felt unwell, began to shiver and vomited blood   On the third day she noticed hæmorrhagic spots on her body and there was bleeding from the gums   She was admitted to hospital where she was found to have large ecchymoses over the body, arms and legs, some as large as 2-3 inches in diameter, and some hæmorrhagic spots in the mouth, on the tongue and inside the lips   There were no other abnormalities on physical examination and the spleen could not be felt   A blood count at that time showed only 9,000 platelets per c mm   but two days later this number had risen to 235,000 per c mm   and the bleeding from the gums had ceased   During the course of the next few days she recovered completely   She said that she had always bruised easily and that her gums sometimes bled when she cleaned her teeth   There was no family history of purpura except that her maternal grandmother had suffered from pernicious anæmia and in the course of this had developed some hæmorrhagic spots on the skin

TABLE I　*Case 1*

Date	RBC per c mm × 10⁶	Hb% (Haldane)	WBC per c mm	Neutrophil polymorphs per c mm	Basophil polymorphs per c mm	Lympho cytes per c mm
22 2 45	5 3					
23 2 45 6 0 a m	Sedormid 0 375 grams given					
8 45 a m	5 25		13,700	12,900		750
10 30 a m						
12 10 p m to 1 40 p m	Transfusion 450 ml fresh Group A blood					
12 25 p m						
2 30 p m	4 56	90				
8 30 p m						
24 2 45 9 0 a m	4 4	90	12,700	11,600	50	450
9 0 p m						
25 2 45 10 0 a m	4 2	84	9,700	8,100	100	1,200
26 2 45 11 30 a m						
6 30 p m						
27 2 45 2 0 p m	3 0	60	11,300	9,400	50	1,150
28 2 45 10 0 a m	2 7					
4 3 45 6 30 p m	3 7	74				
8 3 45	4 2					

N H *Blood counts*

Mono cytes per c mm	Platelets per c.mm	Bleeding time in mins	Coagulation time in mins	
	355 000			
50	Too few to estimate	8 87		A large proportion of the polymorphs show cytoplasmic vacuoles
				Blood obtained from a donor who was subsequently shown to have a normal platelet count and to show no hypersensitivity to sedormid
			2 min 40 secs	Normal coagulation time = 75 secs
	Ditto	77+		
	Ditto	21		
600	Ditto	58+		A large proportion of the polymorphs show cytoplasmic vacuoles
	Ditto	27		
300	Ditto	43		Only an occasional polymorph shows any vacuoles
		16		
		6		
700	66 000	3 5 (at 10 30 a m)		Polymorphs appear normal
	163 000	2 25		
	326 000	2		
	311 000			Patient menstruating—loss normal

Case 3   J MacP , male, born 1887   This patient had been taking sedormid tablets at irregular intervals for about a year   On the morning after he took the last tablet he woke up to find that his tongue was sore and on looking in the glass he saw " blood blisters on the tip "   During the day a few red spots appeared on his face and trunk   On the following morning he found his legs covered with a very large number of minute red spots   There were also a few large "blood blisters" on his hands and forearms   Some of them burst and discharged dark blood   He was admitted on the same day (17 9 45) to University College Hospital under the care of Professor H P Himsworth

On examination there were a few large petechiæ on the face and mucous membranes of the mouth   There were also petechiæ and ecchymoses on the chest and some ecchymoses in the right groin   Several large "blood blisters " were observed on the fingers of both hands, and the legs showed very numerous pin point petechial hæmorrhages   There was no increase in capillary fragility as shown by the venous occlusion technique   Two days after admission (19 9 45) a blood count showed the following findings —

Hb	84% (Haldane)
R B C	4,400,000 per c mm
Reticulocytes	4%
W B C	8,000 per c mm

Differential count

Neutrophil polymorphs	3,500 per c mm
Lymphocytes	3,900 per c mm
Monocytes	600 per c mm
Platelets	44,000 per c mm
Prothrombin index	98%
Bleeding time	4 minutes 10 seconds
Coagulation time	1 minute 50 seconds

No further purpuric hæmorrhages appeared after admission   The patient was discharged 24 9 45, by which time the platelet count had risen to 150,000 per c mm   He has kept well since

Preparation of solutions of sedormid *

The solubility of sedormid in water is only about 1 part in 4,000 at 18°C   To make a saturated solution the drug must be boiled with water for a few seconds and the resulting solution allowed to cool   As it cools, sedormid crystallises out, leaving a solution which, as it tends to be super-saturated, should be left overnight before being filtered   In all the experiments described below solutions of sedormid have been prepared in this way by boiling the drug for a few seconds with either 3 2% sodium citrate $(Na_3C_6H_5O_7, 2H_2O)$ or 0 9% sodium chloride   3 2% sodium citrate was

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* Sedormid is the name of a proprietary tablet containing as its active principle the chemical allyl isopropyl acetyl carbamide   For the sake of brevity, throughout the rest of this paper the term sedormid is used to refer to the active principle except when sedormid tablets are specifically mentioned

used in place of the usual 3 8% solution because the latter causes some hæmolysis after about 2-3 hours if the blood is kept constantly agitated The 3 2% solution, which is isotonic with blood (30), produces no hæmolysis under these conditions for at least 12 hours

Effect of sedormid on platelets

Agglutination of platelets by sedormid Blood was taken from an arm vein in an oiled syringe and 1 0 ml was quickly added to 10 0 ml of a saturated solution of sedormid in 3 2% sodium citrate in a waxed tube As a control, a further 1 0 ml of blood from the same syringe was added to 10 0 ml of citrate alone in a second waxed tube Both tubes were closed with waxed corks and were kept mixed by repeated inversion Samples were removed at intervals and dry films were made which were later stained with Leishman s stain

In Cases 1 and 2 it was found that although the platelets were uniformly distributed over the slides in the citrate preparations they were often collected into small clumps of 2, 3 or 4 and sometimes more platelets in the sedormid preparations The degree of platelet agglutination at different times was therefore estimated by counting 500 platelets in each preparation and expressing the number agglutinated as a percentage of the total number counted The percentage of agglutination of platelets in the control preparations in citrate was also investigated It was found that the agglutination of platelets in the blood of these two patients began as soon as the blood was added to the sedormid solution The percentage agglutinated increased rapidly for the first 15 to 30 minutes and then more slowly until the end of the first hour by which time the agglutination was maximal The percentage of platelets agglutinated then slowly fell over the next 3-4 hours as the total number of platelets slowly decreased This fall in the percentage of platelets agglutinated appeared mainly to be due to separation of platelets from some of the agglutinates This is shown by the following analysis from Case 1 of the platelet agglutination at 1 and 5 hours after the addition of blood to a saturated solution of sedormid in citrate Although the total platelet count was falling during this period the number of free platelets rose from 319 to 486 per 10,000 R B C

1-*hour sample*

    Agglutinated platelets = 500 per 10,000 R B C
    Free platelets = 319 per 10,000 R B C

    Total = 819 per 10,000 R B C

5-*hour sample*

    Agglutinated platelets = 162 per 10,000 R B C
    Free platelets = 486 per 10,000 R B C

    Total = 648 per 10,000 R B C
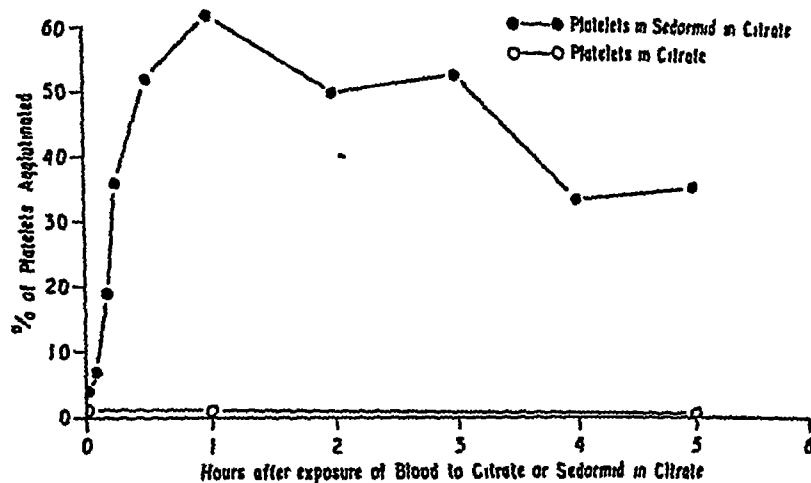
Fig 2   Agglutination of platelets by sedormid in blood from Caso 1 (N H) on 18 8 45   Similar
results were obtained on 28 7 45 and 10 8 45   (See Table III)
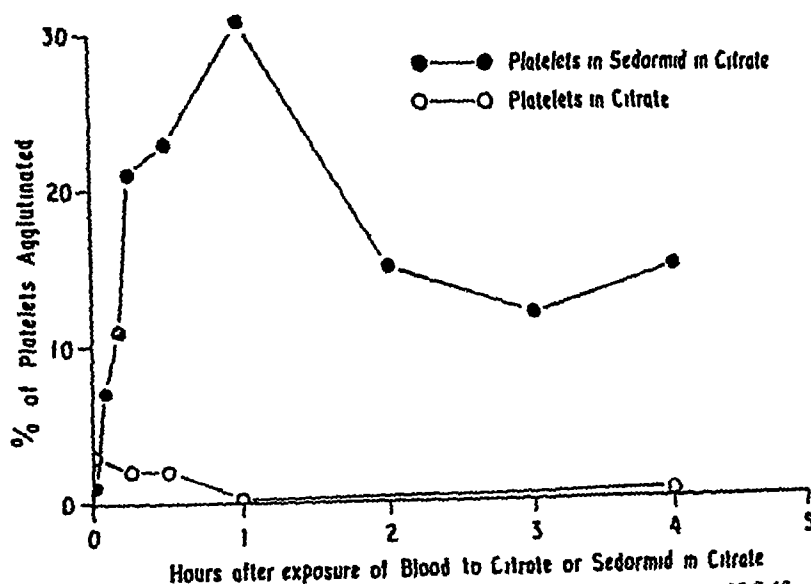
Fig 3   Agglutination of platelets by sedormid in blood from Case 2 (M B) on 26 2 46   A similar
result was obtained on 3 10 46   (See Table IV)
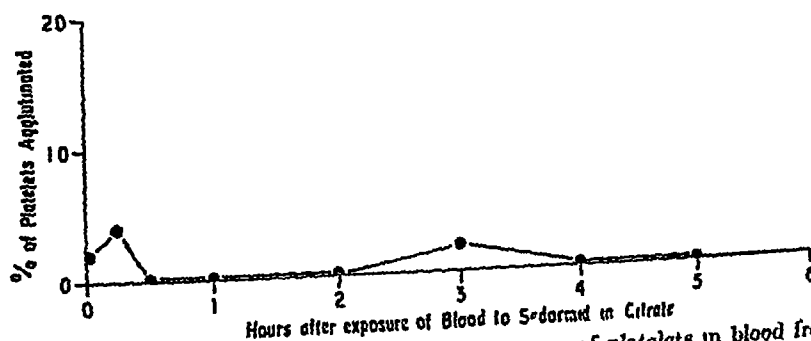
Fig 4   Showing failure of sedormid to cause agglutination of platelets in blood from Case 3
(J MacP) on 11 12 45   Similar results were obtained 7 12 45 and 9 12 45   (See Table V)

TABLE III.

Agglutination of platelets by sedormid in blood from Case 1 (λ H)

Date	Time after blood added to sedormid	% of platelets agglutinated	
		In citrate	In sedormid in citrate
28 7 45	2½ m.ns	0 5	0
	17½ mins		26
	48 mins		41
	1 hr 18 mins	1 0	38
	2 hrs 18 mins		37
	4 hrs 18 mins	1 0	35
	5 hrs 18 mins		30
10 8 45	1½ mins		5
	2½ mins		5
	3¼ mins		8
	4½ mins		7
	6 mins	2	9
	8½ mins		10
	10 mins	2	17
	15 mins	3	29
18 8 45	0 5 mins	1 0	4
	2 mins		1
	3 mins		3
	4 mins		10
	5 mins		7
	6 mins		14
	8 mins		9
	10 mins		19
	15 mins		36
	30 mins		52
	1 hour	1 0	62
	2 hours		50
	3 hours		53
	4 hours		34
	7 hours	0 5	36

At no time did the platelets in the control preparations in citrate alone show any significant agglutination

In Case 3, who was a much milder case than the other two, it was not possible to demonstrate any agglutination of platelets by sedormid   The findings in these three patients are shown in Figs 2, 3 and 4, and in Tables III, IV and V

Eight patients with unrelated diseases were investigated in the same way   In none was there any significant difference between the percentage of platelets agglutinated in the sedormid preparations and those in citrate alone   The findings at the end of one hour are shown in Table VI

Survival of platelets in sedormid   The platelets in the same preparations were later counted by the method of Fonio (27), the results being expressed as the numbers of platelets per 1,000 red cells   In the absence of hæmolysis

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TABLE IV

Agglutination of platelets by sedormid in blood from Case 2 (M B)

</div>

Date	Time after blood added to sedormid	% of platelets agglutinated	
		In citrate	In sedormid in citrate
26 2 46	1 min	3	1
	2 mins		6
	3 mins		3
	5 mins		7
	10 mins		11
	15 mins	2	21
	30 mins	2	23
	1 hour	0	31
	2 hours		15
	3 hours		12
	4 hours	0 5	15
3 10 46	5 mins	3	8
	15 mins		6
	30 mins		11
	1 hour	5	17
	2 hours		13
	3 hours		14
	4 hours	2	16

any change in the ratio of platelets to red cells in the two tubes will indicate an alteration in the number of platelets   Counts were performed as soon as possible after the tubes had been set up and then after 4-5 hours, by which time the percentage of agglutinated platelets had fallen sufficiently low to make accurate platelet counting possible while morphological changes had not become so great as to make the platelets difficult to identify   In order to

TABLE V

Agglutination of platelets by sedormid in blood from Case 3 (J MacP)

Time after blood added to sedormid	% of platelets agglutinated by sedormid in citrate		
	7 12 45	9 12 45	11 12 45
10 mins	4	0	*r*
15 mins	0	0	4
30 mins	0	0	0
1 hour	0	0	0
2 hours	0	0	0
3 hours	0	4	2
4 hours	0	0	0
5 hours	4	0	0

TABLE VI

Effect of sedormid on platelets
Controls

Patient	Sex	Diagnosis	% agglutination of platelets at 1 hour	
			Citrate	Sedormid in citrate
W	F	Pregnancy—very mild hæmolytic anæmia	0 5	1
M	M	Healthy man	—	1
H	F	Healthy woman	—	0 5
J	J	Osteo arthritis	5	5
BR	M	Carcinoma of bronchus	5	5
H	M	Myocardial infarction	5	4
W	F	Chronic cholecystitis	—	1
WO	M	Bronchiectasis	2	4

obtain comparable figures it was found necessary to count between 5,000 and 10,000 red cells in each preparation   The counts obtained in each of the three patients are shown in Table VII   As Wright (87) has shown, the platelet counts in such preparations may be expected to fall slowly   In these experiments this same phenomenon was observed   There was however no difference in the rate of disappearance of the platelets in citrate from that observed in a saturated solution of sedormid in citrate and the platelet counts at the end of 4-5 hours were, within the limits of experimental error, identical   It was therefore concluded that sedormid causes a specific agglutination of platelets in the blood of some patients who have recovered from sedormid purpura but that under the conditions of these experiments no lysis of platelets occurs

TABLE VII

Platelet survival in sedormid in citrate

Patient	Date	Duration of experiment	Platelet counts (Platelets per 1,000 R B C)			
			Beginning of experiment		End of experiment	
			Citrate	Sedormid in citrate	Citrate	Sedormid in citrate
NH*	18 8 45	5 hours	87	88	68	65
MB*	26 2 46	4 hours	77	77	58	56
J MacP†	11 12 46	4 hours	52	52	43	43

* 10,000 R B C were counted for each platelet count
† 5,000 R.B C were counted for each platelet count

Effect of sedormid on clot retraction

It has been repeatedly demonstrated that, if platelets are removed from plasma by centrifugalisation, then clot retraction is abolished (3, 47, 72, 79, etc)   This has been confirmed on many occasions during the present investigation   The retraction of plasma clots has invariably been reduced from about 90% to less than 4% by centrifuging off the platelets   A typical result is shown in Fig 5   This same effect is produced if the platelets are destroyed in vivo or in vitro by the action of a potent anti-platelet serum (79)   It was therefore argued that if sedormid had an effect on platelets, the addition of sedormid to the blood of a patient who had recovered from purpura due to this drug might diminish its clot retraction

It was found that a diminution in clot retraction could be consistently produced by the addition of a saturated solution of sedormid in saline to the blood of each of the three patients described above   A typical result

is shown in Fig 6   To estimate this reduction in clot retraction quantitatively 2 0 ml of blood were added to 0 5 ml of a saturated solution of sedormid in saline in a clot retraction tube   The tube was then corked and the contents mixed by repeated inversion   Three or four tubes were usually set up for each investigation   An equal number of control tubes in which saline was used in place of the sedormid solution were also set up   The clot retraction in the two sets of tubes was measured by a method described elsewhere (1) No correction was made for the hæmatocrit reading as this was constant for

TABLE VIII

Inhibition of clot retraction by sedormid

Patient	Date	Saline		Sedormid in saline	
		No of experiments	% clot retraction	No of experiments	% clot retraction
NH	29 5 45	3	74	4	38
	28 7 45	2	65	2	34
	10 8 45	5	69	5	15
	21 5 46	2	63	2	12
	18 6 46	4	73	4	22
	25 6 46	2	72	2	6
	12 9 46	4	68	4	38
MB	26 2 46	4	64	4	5
	13 4 46	4	58	4	2
	9 8 46	4	64	4	2
	21 9 46	3	65	3	7
	28 9 46	3	64	10	15
J MacI	7 12 45	5	64	5	29
	14 10 46	4	68	3	45
	16 10 46	4	67	3	50
	6 11 46	4	66	4	47

alone, should be at least three times the standard error of the difference between the observations, *i e*, 6 0% As all the figures in Table VIII are based on multiple observations even smaller differences will be significant In fact the smallest difference observed was 17% and in most cases the difference was much greater, thus proving that in each of the three patients examined sedormid causes a statistically highly significant reduction in clot retraction That this is not an effect of sedormid on normal blood can be seen from the results obtained in a series of 18 controls of blood from patients with unrelated diseases In no case was there a difference of more than 3% between the clot retraction of blood diluted with a solution of sedormid in saline and that observed when saline alone was used as a diluent The figures obtained with these controls are shown in Table IX and are represented diagrammatically in Fig 7
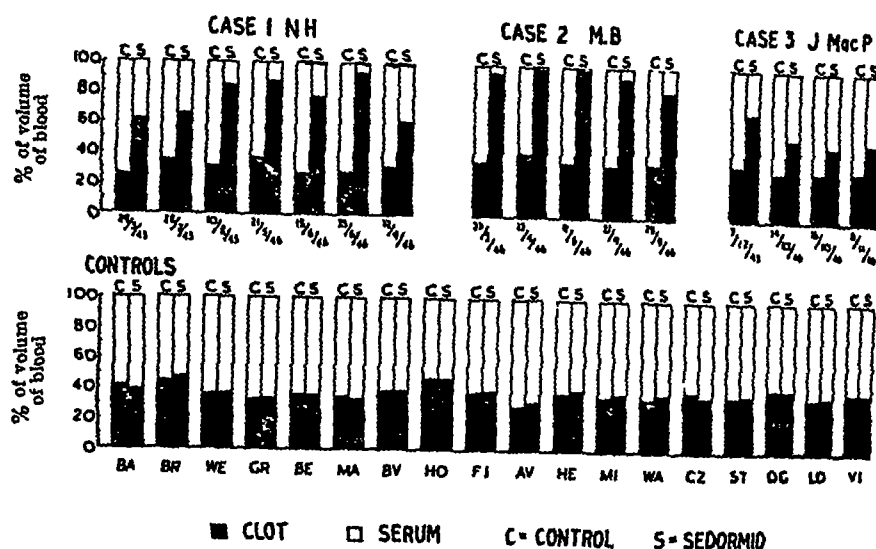
Fig 7 Diagram showing effect of sedormid on clot retraction of three patients who had recovered from sedormid purpura and of a series of eighteen controls

The observation that sedormid causes agglutination of platelets in the blood of patients who have recovered from sedormid purpura makes it very probable that the reduction in clot retraction which has been observed in these patients is due to an effect of sedormid on their platelets

Investigation of the concentration of sedormid required to reduce clot retraction in vitro

The solubility of sedormid in 0 9% sodium chloride is 0 025% (mean of three determinations) The minimum concentration of sedormid required to reduce clot retraction has been investigated to determine whether such concentrations may be expected to occur in the body after the administration of therapeutic doses of the drug The results are shown in Table X, from which it will be seen that a statistically significant reduction in clot retraction is produced by a saturated solution of sedormid in 0 9% sodium chloride

diluted 1   10 with 0 9% sodium chloride when this is mixed with blood in the proportion of 0 5 ml of sedormid solution to 2 0 ml of blood to produce a final concentration of 0 0006%   Even if sedormid is distributed uniformly throughout the total intra and extracellular body fluid which in a normal

TABLE IX

Effect of sedormid on clot retraction in blood of patients with no acquired hypersensitivity to sedormid

Patient	Sex	Diagnosis	Saline		Sedormid in saline	
			No of experiments	Clot retraction %	No of experiments	Clot retraction %
BA	M	Cerebral tumour	5	58	5	61
BR	M	Carcinoma of bronchus	4	55	4	52
WE	M	Thrombophlebitis migrans	4	64	4	63
CP	M	Malignant hypertension	4	67	4	66
BF	M	Essential hypertension	3	64	3	64
MA	M	Essential hypertension	4	65	4	66
BA	M	I V failure	4	61	4	61
HO	M	Cerebral thrombosis	4	53	4	53
FI	F	Healthy woman	4	62	4	61
AV	F	Pregnancy — very mild hæmolytic anæmia	5	71	5	68
HI	F	Nephritis — apparently healed	4	62	4	60
MI	I	Congestive cardiac failure	4	64	4	62
WA	F	Pleural effusion	4	65	4	62
CZ	F	Essential hypertension	4	60	4	63
NI	I	Hypertension	4	63	4	62
Ot	I	Congestive cardiac failure	4	55	4	58
TO	F	C P I	4	64	4	63
VI	F	Thyrotoxicosis	4	60	4	60

TABLE X

The effect of lowering the concentration of sedormid on its power to reduce clot retraction in the blood of a patient (MB) who had recovered from purpura due to sedormid

Solution used for diluting blood	No of experiments	Mean clot retraction % (0 5 ml diluent + 2 0 ml blood)
Saline	4	03
Saturated solution of sedormid in saline = A	4	2
Solution A diluted 1 10 with saline	3	54
Solution A diluted 1 100 with saline	4	61
Solution A diluted 1 1,000 with saline	4	62

adult has a volume of 49 litres (51) then 0 5 gram (2 tablets) of sedormid would, if fully absorbed, give a final concentration of 0 001% in the body fluids This concentration is highei than that required to reduce clot retraction in vitro Moreover it is probable that tempoiarily, befoic diffusion has occurred into the extravascular fluids, much highei concentrations of the drug will be achieved in the blood As it is probable that the reduction in clot retraction is due to an effect of sedormid on the platelets this finding would seem to indicate that a deleterious effect on platelets may well be produced in the body by therapeutic doses of the drug

Effect of sedormid on the capillaries

It has been repeatedly shown that thrombocytopenia alone is not sufficient to produce purpura and that there must also be an associated capillary lesion before purpuric haemorrhages can occur The above experiments show that sedormid has an effect on the platelets of patients who have recovered from purpura due to this drug The following work was undertaken to demonstrate a further and separate effect of sedormid on the skin capillaries

Intradermal testing with sedormid Injections of sedormid in saline were given intradeimally into the volar surfaces of the forearms of Cases 2 and 3 No local haemorrhages were produced by the injections nor was there any suggestion of an urticarial reaction different from that produced by saline alone The details of these tests are shown in Table XI Case 3 showed no reactions of any kind following a single intradermal injection of 0 1 ml of a saturated solution of sedormid in saline Case 2 was injected in two places on the left forearm at 11 0 a m , 9 8 46, with 0 05 ml and 0 1 ml of a 1 1000 dilution of a saturated solution of sedormid in saline At 4 0 p m on the same day there were no changes to be seen at the sites of the injections and a furthei intradermal injection of 0 05 ml of a 1 100 dilution of sedormid in

TABLE VI

Results of intradermal testing with sedormid

Patient	Date	Amount injected	Control	Results Control	Results Sedormid
Case 2 VH	9 4 48 11 0 a m	0 05 and 0 1 ml of 1 1000 dilution of saturated solution sedormid in saline	0 05 and 0 1 ml saline	No haemorrhages	No haemorrhages
	9 4 48 5 0 p m	0 05 ml 1 100 dilution of saturated solution sedormid in saline	0 05 ml saline	No haemorrhages	No haemorrhages
	10 4 48 10 30 a m	0 05 ml 1 10 dilution of saturated solution sedormid in saline	0 05 ml saline	No haemorrhages	No haemorrhages
	10 4 48 10 30 a m	Platelet count 73 000/c mm V 18 Platelets numerous 10 50 a m, 0 8 48			
	10 8 48 10 0 p m	Platelet count 502,000/c mm			
		Bleeding time (Duke) 4 min			
		Capillary fragility (positive pressure method) normal			
	11 8 48	Mild attack of purpura			
Case 3 J Mack	16 1 48	0 1 ml saturated solution of sedormid in saline	0 1 ml saline	No haemorrhages No increase in capillary fragility	No haemorrhages No increase in capillary fragility

saline was given into a third site on the same forearm   There was no immediate reaction   The patient returned to hospital on the following morning   As there were still no local changes at the sites of the injections, she was given 0 05 ml of a 1 10 dilution of a saturated solution of sedormid in saline, again into the left forearm   Twenty minutes later there were still no local changes in the skin and blood was taken by venepuncture for another investigation   The platelet count, performed on this blood after she had left the hospital, was only 33,000 per c mm   (Gram's (31) method) The patient returned to the hospital at 10 0 p m that evening and appeared perfectly well   The capillary fragility as estimated by a positive pressure method was not increased even at the sites of the intradermal injections The bleeding time by Duke's method (18) was 4 minutes, and the platelet count by Gram's method was 502,000 per c mm   In spite of these normal findings, the patient developed a mild attack of purpura during the night, and on the following morning there were haemorrhages on the inner aspect of the right arm above the elbow and around the waist   The lobe of the left ear, where it had been punctured for estimating the bleeding time on the previous evening, had become black and swollen   About midday the patient began to menstruate   This menstrual period occurred at the expected time but the loss was unusually great   Most unfortunately the patient, who is a doctor and whose description of her attack of purpura can be relied upon implicitly did not report this attack for several days and in consequence no further platelet counts were performed   It is very difficult, if not impossible, to establish from the data available, the true course of events that led up to this attack of purpura   The normal platelet count observed on the night before the purpura appeared would suggest that the haemorrhages were a delayed effect of the final injection of sedormid and that the thrombocytopenia noted 20 minutes after this injection may have been due to the previous injections   Whatever the explanation of this attack of purpura, however, it is clear that it followed an almost incredibly small dose of sedormid as the total amount injected over the whole three day period was only 1 4 $\times$ 10^{-6} grams

Patch testing with sedormid   Although the intradermal injection of sedormid failed to produce any local lesion in these patients the application to the skin for 24-48 hours of a suspension of sedormid crystals in a saturated solution in a suitable solvent caused petechial haemorrhages to appear in the area to which the preparation was applied   There was no local hyperaemia or wheal formation to suggest that the haemorrhages were due to release of histamine nor was there any rise in capillary fragility in other parts of the body or any significant alteration in the platelet count   No haemorrhages were produced when the solvents alone were used as controls   The results obtained from patch testing are summarised in Table XII

In the earlier experiments, which were performed on Case 1, decinormal sodium hydroxide was used as a solvent   On the first two occasions on which

)atch testing was successfully performed the patches were applied in the ifternoon and were removed on the following day On each occasion)urpuric hæmorrhages were produced in the test area A few days later he test was repeated using an identical technique On this occasion, no iæmorrhages were produced but after congestion of the arm by a cuff at a)ressure of 80 mm of mercury for 5 minutes, the test area became closely)acked with small petechiæ while no petechiæ were produced in the surround-ng skin Two further patches were applied on that morning using acetone is a solvent They were left on for 48 hours and on this occasion numerous purpuric hæmorrhages were produced in both test areas This increase of resistance of the skin capillaries to the action of sedormid may have been due to the fact that the patient was menstruating at the time of the two earlier experiments It has been shown (7, 74) that capillary fragility is considerably increased at the time of the menstrual period and Moeschlin (54) has described a woman who developed severe and extensive hæmorrhages if she took sedormid immediately before her menstrual period At other times, however, she developed only very mild purpura after taking the drug

By far the most satisfactory results from patch testing were obtained when propylene glycol was used as a solvent The sedormid was made to dissolve by gentle heating As the solution cooled crystals of sedormid separated out making a thick paste which was spread on an area of skin which had previously been cleaned with ether or acetone The paste was covered with a piece of filter paper backed with oiled silk and the whole held in place with a wide strip of elastoplast A control using propylene glycol alone was set up in a similar manner Throughout these investigations the patch has been applied to the skin of the inner side of the arm about 5 cms above the bend of the elbow as the skin in this area has been stated to be more readily permeable than it is in most other parts of the body (81)

The photographs (Fig 8) show the result of patch testing Case 1 with sedormid using propylene glycol as a solvent Similar but less striking results were obtained in Case 2 but in Case 3, a much less severe case of purpura, the results were consistently negative In this patient patch and scratch testing with sedormid dissolved in acetone and decinormal sodium hydroxide and also patch testing with sedormid in propylene glycol failed to produce either local hæmorrhages or any significant increase in capillary fragility Negative results with sedormid in propylene glycol have also been obtained in a series of 20 controls, 10 men and 10 women, suffering from unrelated diseases In none of these patients were any hæmorrhages produced in the test areas In two, however, the patches produced a slight inflammatory reaction with some desquamation of the superficial epithelium This appeared to be due to the propylene glycol as the same effect was noted in the control areas which had been in contact with propylene glycol alone

TABLE XII   *Patch*

Date	Solvent	Duration of experiment	Patch	Control
Case 1 N H 13 4 45 N B	N/10 NaOH Patient menstruating	18 hours	Purpuric hæmorrhages + +	Negative
14 4 45 N B	N/10 NaOH Patient menstruating	26 hours	Purpuric hæmorrhages + +	Negative
20 4 45	N/10 NaOH	24 hours	No hæmorrhages, but capillary fragility increased greatly in test area	Negative
21 4 45	Acetone (2 patches)	48 hours	Purpuric hæmorrhages in both areas	Negative
10 9 46	Propylene glycol	48 hours	Very numerous hæmorrhages over whole area of patch	Negative
Case 2 M B 26 2 46	Propylene glycol	48 hours	Purpuric hæmorrhages +	Negative
Case 3 J MacP 7 12 45	Acetone and N/10 NaOH	48 hours	No hæmorrhages	Negative
9 12 45	Acetone and N/10 NaOH SCRATCH TEST	48 hours	No hæmorrhages	Negative
14 10 46	Propylene glycol	48 hours	No hæmorrhages	Negative

testing with sedormid

Investigations performed immediately after removal of patch

Platelets = 360 000/cu.mm
Capillary fragility (negative pressure method) — on other arm, no increase

Platelets = 250 000/cu mm
Capillary fragility (positive pressure method)
   Test arm — petechiae from band to junction of middle and upper thirds of forearm
   Control arm — No petechiae

Platelets — numerous in stained films
Capillary fragility (positive pressure method)
   Test arm — no increase in fragility except in area to which sedormid patch applied
      This area became closely packed with small petechiae
Clot retraction   — 75%

Platelets ~ 610 000 cu mm
Capillary fragility (negative pressure method) — on both forearms, not increased
Clot retraction   ~ 68%

Platelets — numerous in stained films
Capillary fragility (positive pressure method)
   Test arm — no increase in fragility except in area to which sedormid patch applied
      This area became filled with small petechiae
   Control arm — no increase in fragility
Clot retraction   61%

Platelets   numerous in stained films
Capillary fragility (positive pressure method)
   Test arm — about 20 petechiae in test area and about 4 cms around in all
      directions   Result probably not significant
   Control arm — no haemorrhages

The observation that the application of sedormid to the skin can cause local hæmorrhages in the absence of any general increase in capillary fragility or lowering of the platelet count would seem to prove that sedormid has, in addition to its effect on the platelets, a specific effect on the capillaries, at least in some patients who have recovered from sedormid purpura Why this effect should be demonstrable on patch testing and yet not by the intradermal injection of sedormid is not at all clear One patient developed generalised purpura after intradermal skin testing and yet failed to develop local purpura at the site of the injections It is clear therefore that the failure to produce local hæmorrhages was not due to inadequate dosage It is, however, possible that the saline solution of sedormid given intradermally was removed by the lymphatics too rapidly for it to have any demonstrable local effect and that the sedormid in the patch test was able to cause local hæmorrhages because the skin capillaries were in contact with the drug for a much longer period Some estimate of the time required for sedormid to act on the skin capillaries is provided by the following observations made on Case 1 (N H) Firstly, it was found that on one occasion, a patch left on for 24 hours produced no hæmorrhages whereas two patches applied to the same patient on the following day and left on for 48 hours produced numerous hæmorrhages (see Table XII) Secondly, when this patient was given a test dose of sedormid by mouth, cutaneous hæmorrhages did not appear for 8 hours and were not really extensive until the following day, although she began to vomit blood and to pass blood per vaginam within 30 minutes of taking the drug It appears therefore that sedormid requires a much longer time to act on the cutaneous vessels than it does on those supplying mucous membranes If it is assumed that a minimum period of 8 hours is required for sedormid to act on the skin capillaries it seems probable that all the drug given in an intradermal test will have been removed from the site of injection long before it has had time to produce any local hæmorrhages This may well explain the apparent anomaly of the failure of intradermal testing with a dose of sedormid sufficient to cause generalised purpura

Attempts at passive transfer of sedormid hypersensitivity

Forty subjects have been injected intradermally with 0 05 to 0 3 ml of freshly prepared serum from Cases 1 and 2 in an endeavour to produce passive transference of hypersensitivity to sedormid by the Prausnitz-Kuestner (61) technique Sedormid has subsequently been given to these individuals by mouth, by injection of a saturated solution in saline into the area previously injected with serum or by the application of patches of sedormid in propylene glycol to this area The interval between the injection of serum and the administration of sedormid has been varied from a few minutes to as long as 10 days The area of skin injected with serum has been inspected and the capillary resistance estimated at frequent intervals up to 96 hours after the

drug has been given   In no case has any significant change been noted in the skin nor has there been any increase in capillary fragility   These uniformly negative results are in accordance with those of most other workers and as has already been stated, there is no satisfactory evidence that this type of hypersensitivity has ever been transferred to another individual

Sensitivity to other open chain ureides

Sedormid appears to be the only member of the open chain ureide series of hypnotics which ever causes purpura   These compounds are so closely related chemically that it might be expected that sensitisation to one would be associated with sensitisation to other members of the series Such a group sensitisation has been observed in purpura due to the arsenobenzol compounds (21), and the sulphonamides (16, 35), though not to the open chain ureides (32)   To investigate this matter further it was decided to observe the effect of adalin and bromural on the clot retraction of the blood of two patients who had recovered from purpura due to sedormid

The solubility of sedormid in saline is 0 025%   The effect of 0 033% adalin and 0 044% bromural in saline on clot retraction was investigated in the same way as that used to investigate the effect of sedormid on clot retraction, 0 5 ml of the solution being mixed with 2 0 ml of blood, the mixture then being allowed to clot   Controls, using saline as a diluent were put up at the same time

In Case 1 (N H) each of the two drugs produced a statistically significant reduction in clot retraction, the effect with adalin being considerably greater than that obtained with bromural   In Case 2 (M B), only adalin produced any significant reduction in retraction   No such reduction with either adalin or bromural was observed in samples of blood taken from eight patients who had no acquired hypersensitivity to sedormid

TABLE XIII

The effect on clot retraction of the addition of adalin or bromural to the blood of patients who have recovered from sedormid purpura

Patient	Date	Clot retraction with saline added		Clot retraction with adalin in saline added		Clot retraction with bromural in saline added	
		No of experiments	Mean clot retraction %	No of experiments	Mean clot retraction %	No of experiments	Mean clot retraction %
N H	20 5 47	4	73	4	55	4	65
M B	3 10 46	4	66	4	54	4	64
	21 10 46	4	69	4	59		
	10 5 47	4	63	4	54		

It would therefore appear, in spite of Hadorn's (32) negative findings, that the administration of other open chain ureides to patients who have recovered from purpura due to sedormid is probably unwise   The results of these investigations are shown in Tables XIII and XIV

TABLE XIV

The effect on clot retraction of the addition of adalin or bromural to the blood of patients who had no acquired hypersensitivity to sedormid

Patient	Diagnosis	Clot retraction with saline added		Clot retraction with adalin in saline added		Clot retraction with bromural in saline added	
		No of experiments	Mean clot retraction %	No of experiments	Mean clot retraction %	No of experiments	Mean clot retraction %
DD	Gonococcal arthritis	4	54	3	51	3	54
JF	Ulcerative colitis	4	50	4	58	4	58
GN	Coronary thrombosis (healed)	4	55	4	53	4	53
JO	Healthy woman	4	60	4	60	4	61
MF	Healthy woman	3	70	4	70	4	68
RB	Spontaneous pneumo thorax	4	64	4	63	4	62
PR	Pheno barbitone poisoning	4	60	3	58	4	60
HS	Hypertension	4	61	4	59	4	58

Value of these results in diagnosis

The foregoing work has shown that the addition of sedormid to the blood of patients who have recovered from purpura due to this drug causes a marked reduction in clot retraction and sometimes also, agglutination of platelets and that, furthermore, patch testing with the drug may give rise to local cutaneous hæmorrhages

Cases of thrombocytopenic purpura of sudden onset and short duration are not very uncommon   If the patient has been taking a drug before the purpura developed it is desirable to try to establish a causal relationship between the drug and the attack of purpura   Hitherto, the only way in which this could be done has been to give a test dose of the drug, a procedure which, as in Case 1, may produce quite alarming reactions   If sedormid is suspected as the cause of an attack of purpura, at least the effect of this substance on clot retraction should be investigated and a test dose of sedormid should only be given if this investigation gives negative results   The following case history illustrates the value of such investigations

Case 4   (E F)   female, born 1877   For about a year this patient had been feeling tired with lack of appetite and loss of weight   She had been given ascorbic acid by her doctor   In March, 1943, she started to take sedormid   She took about 12 tablets over a period of 4 weeks She then took no more until August, 1943, when she took three further tablets   Four days later (22 8 43) she noticed a rash on her feet and after a further five days she was admitted to

hospital (27 8 43) when she was found to have profuse small purpuric spots on the feet and legs and large hæmorrhagic patches on the trunk, neck, face and arms. There were ecchymoses on the tongue and buccal mucosa. The spleen was not palpable. On the following day a blood count showed a severe orthochromic anæmia and the platelets numbered less than 2 500 per c mm. Two days later she was transfused with one pint of fresh blood as the hæmoglobin had now fallen to 34%. A blood count done after the transfusion showed that the platelet count had risen to 100 000 per c mm. The urinary excretion of ascorbic acid was found at this time to be within normal limits, the concentration varying from 1 0 to 22 0 mg per 100 ml urin. After the transfusion her general condition rapidly improved and the purpura began to fade. By 6 9 43 the hæmorrhages had almost disappeared and the platelet count had risen to 250 000 per c mm. Thereafter she made a steady recovery.

She was next seen 7 2 46 when she was referred for investigation as a case of transient thrombocytopenic purpura probably due to sedormid. Skin tests using acetone and decinormal sodium hydroxide as solvents were put up for 24 and 72 hours. No local hæmorrhages resulted and the capillary fragility as estimated by a positive pressure method was not increased in the test areas. The platelets showed no significant agglutination (maximum 1 0%) when 1 0 ml of blood was added to 10 0 ml of a saturated solution of sedormid in isotonic sodium citrate. In addition of a saturated solution of sedormid in saline to this patient's blood caused no inhibition of clot retraction. The figures obtained (each being the average of four estimations) were as follows

Clot retraction of 2 0 ml blood + 0 5 ml saline = 62%

Clot retraction of 2 0 ml blood + 0 5 ml sedormid in saline = 61%

In view of these negative findings it was decided to admit the patient to hospital for test dosing with sedormid. At 8 30 p m 14 2 46, she was given ½ tablet of sedormid (0 03 gm). She experienced no reactions and there was no increase in capillary fragility. On the following evening she was given one tablet (0 25 gm) of sedormid with similarly negative results. It is clear therefore that whatever the cause of her attack of purpura in 1943 this patient was not, at the time she was examined suffering from hypersensitivity to sedormid, a conclusion which could not have been arrived at without the aid of the various tests employed as without them test dosing would have been considered far too dangerous a procedure in such a frail old woman.

have shown that a sudden lowering of the platelet count may precipitate haemorrhages in animals whose capillaries have been damaged to an extent insufficient to cause purpura   Bedson found that this degree of capillary damage could be produced by the administration of anti-red cell serum to guinea pigs   When he gave the same animals " agar serum," which by itself causes a transient fall in the platelets but no purpura, then the animals developed extensive haemorrhages   Further evidence that the platelets are concerned with the maintenance of normal capillary permeability has been provided by Danielli (13) who has shown that the rate of development of œdema in the perfused hind limbs of the frog can be greatly reduced by the addition of mammalian platelets to the perfusion fluid   He considered that the action of the platelets was largely mechanical, involving simple blockage of protein permeable pores in the capillaries

In the present investigation it has been shown that the application of sedormid to the skin of some patients who have recovered from purpura due to this drug results in the appearance of petechial haemorrhages in the area to which the drug is applied   This occurs in the absence of any significant change in the platelet count   Although it is conceivable that diffusion of sedormid through the capillary wall into the blood stream might cause some local destruction of platelets too slight to produce a significant fall in the platelet count elsewhere in the body it seems very unlikely that even if such a local destruction of platelets did occur it could have any effect on local vascular permeability as the blood flowing through the vessels would bring with it a continual supply of fresh platelets   It therefore seems clear that in so far as sedormid purpura is concerned the capillary lesion can occur independently of the development of thrombocytopenia   It has also been shown that sedormid has an effect on the platelets of these patients   This is demonstrated by the production of platelet agglutination by sedormid   The reduction in clot retraction which occurs when sedormid is added to the blood of these patients is probably also evidence of an effect of sedormid on the platelets   As the action of sedormid on platelets occurs in vitro it seems legitimate to infer that thrombocytopenia in sedormid purpura occurs as a separate phenomenon from the capillary lesion   These experimental findings therefore seem to be a clear vindication of the view originally propounded by Nolf (57) and since supported by a number of writers (6, 10, 20, 60, 62, 67, etc), that in thrombocytopenic purpura there are two separate conditions, a capillary defect and a deficiency in the number of circulating platelets   It is probable that the thrombocytopenia increases the haemorrhagic tendency due to the capillary lesion

SUMMARY

1   Three cases of transient thrombocytopenic purpura due to hypersensitivity to sedormid are described   After recovery, purpura was induced again in two of these cases by administration of the drug, in one by as little as $1 4 \times 10^{-6}$ grams of sedormid

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Fig. 5   The effect on clot retraction of the removal of platelets from citrated plasma by centrifugalisation
(a) Plasma clotted with thrombin after centrifugalisation   Clot retraction negligible
(b) Normal plasma clotted with thrombin   Clot retraction 93% (normal)
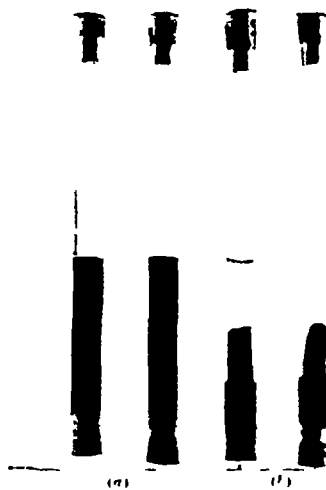
Fig. 6   The effect on clot retraction of the addition of sedormid to the blood of a patient (M B) who had recovered from purpura due to sedormid
(a) Blood plus a saturated solution of sedormid in saline   Clot retraction negligible
(b) Blood plus saline   Clot retraction normal
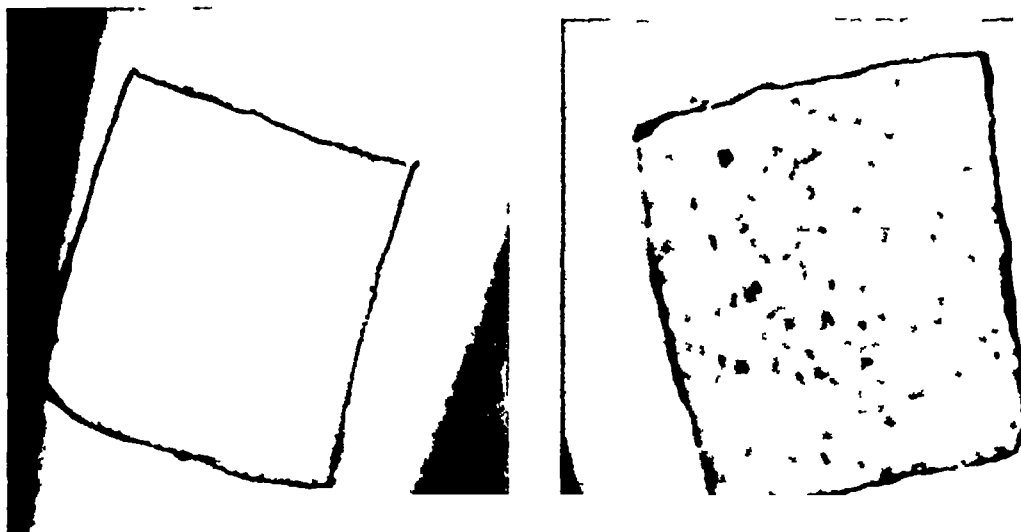
LIVER AND MUSCLE GLYCOGEN IN NORMAL SUBJECTS, IN DIABETES MELLITUS AND IN ACUTE HEPATITIS

PART I UNDER BASAL CONDITIONS

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THERE are very few observations on hepatic and muscle glycogen value in man It seemed of interest to record glycogen distribution in diabetes and acute hepatitis compared with that in normal subjects This paper establishes the range found under basal conditions which was investigated as a preliminary to a study of the changes effected by adrenaline (6)

Methods and material

The normal subjects were hospital patients convalescent from illnesses believed not to affect carbohydrate metabolism

The twelve patients with diabetes had had no insulin for at least two days, or longer if they were previously taking zinc-protamine insulin

Two patients with acute hepatitis were of severe grade, one was mild (10)

Muscle and liver biopsies

The muscle and liver biopsies were each divided into three parts   One portion was fixed in absolute alcohol for section and staining by Best's carmine method for glycogen   The other two were dropped into weighed tubes containing 2 ml 30% potassium hydroxide solution and transferred to the laboratory in a thermos flask containing ice and salt

The tubes were reweighed as soon as possible and the tissue then dissolved by warming on a water bath , 2 ml 95% ethanol were added and the tubes replaced in a boiling water bath till the ethanol boiled and a cloudy precipitate formed (4)   After standing overnight in a refrigerator the tubes were centrifuged hard for ten minutes and well drained   2 ml 50% ethanol were then added and the precipitate dispersed with shaking and tapping   Centrifuging and washing with 50% ethanol were repeated until the supernatant was no longer alkaline to phenolpthalein (2)   The last traces of ethanol were removed by warming, and the precipitate then dissolved in 2 ml 0 6 N perchloric acid   The tubes were then heated in a boiling water bath for 2½ hours to hydrolyse the glycogen to glucose   This was done in an all-glass apparatus, namely, graduated centrifuge tubes with ground glass stoppers to which air condensers were fused   When the tubes were cool the acid was neutralised with 2 0 ml 0 6 N KOH (accurately standardised against the acid used for the hydrolysis), and the white crystalline precipitate of potassium perchlorate spun down   0 5-1 0 ml of the supernatant was used for sugar estimation, and the glycogen content of the tissue calculated

Each sample of muscle used for analysis weighed 15-40 mg , and each sample of liver 8-20 mg

RESULTS

Hepatic glycogen concentration

(*a*) *Normal subjects*   In nineteen patients the hepatic glycogen content was between 0 95 and 4 1% (w/w), mean 2 15, (Table I)   In all these subjects hepatic histology, apart from an occasional fat droplet contained in a liver cell, was normal

(*b*) *Diabetes*   In ten diabetics the range of hepatic glycogen was also very wide, range 0 88-2 98%, mean 1 81, (Table II)   Although the mean was less than for normal subjects a statistically significant difference was not established   The clinical type of diabetes could not be correlated with the hepatic glycogen content   One patient in diabetic coma with a plasma alkali reserve of 10 ml 100 ml and a blood sugar of 408 mg /100 ml gave a hepatic glycogen value of 1 26%

Hepatic histology was usually normal   In five instances glycogenic infiltration of the hepatic cell nuclei was seen , this is a frequent finding in diabetes, but is also occasionally seen in normal subjects   In two patients an occasionally fatty droplet was seen in the liver cells   These were not

outside the bounds of normality   The liver of the patient with diabetic coma showed gross swelling of the liver cells   Nuclear glycogen and fat were not present

TABLE I

Hepatic glycogen in hospital patients under basal conditions

Case	Sex and age		Diagnosis	Hepatic glycogen g per 100 g
J M	F	21	Duodenal diverticulosis	4 0
J B	M	73	Chronic bronchitis	4 1
M H	F	67	Chronic bulbar palsy	3 5
W W	M	50	Convalescent gastritis	3 4
J B	M	45	Convalescent duodenal ulcer	2 9
J H	M	32	Convalescent gastritis	2 5
P A	F	22	Dermatitis	2 15
A P	M	47	Carcinoma of lung	2 06
A B	M	50	Convalescent duodenal ulcer	2 05
J W	M	32	Convalescent gastric ulcer	1 95
J P	M	65	Angina pectoris	1 91
T I	M	24	Idiopathic epilepsy	1 80
J O	M	66	Carcinoma of colon	1 72
B W	M	34	Convalescent bronchopneumonia	1 47
L M	M	34	Idiopathic epilepsy	1 20
S V	M	35	Asthma	1 1
V H	M	44	Convalescent pneumonia	1 1
J V	M	50	Hageman's disease of bone	0 05
J J	M	21	Psychoneurosis	0 05
			Mean	2 1

TABLE II

Hepatic glycogen in diabetes

Case	Sex and age	Blood glucose mg per 100 ml	Alkali reserve mg per 100 ml	Hepatic glycogen g per 100 g
A W	F 60	164	55	2 9b
Z H	F 59	210	—	2 55
J L	M 67	240	—	2 36
M D	M 24	264	49	2 15
R C	F 62	300	57	1 72
J T	M 67	260	59	1 63
W T	M 64	250	—	1 36
G C	M 67	408	10	1 26
L D	M 24	264	55	0 88
C R	M 29	508	—	1 49
			Mean	1 84

TABLE III

The effect on muscle and liver glycogen of repeated biopsies

Case	Sample	Time interval	Glycogen g per 100 g		
			1	2	3
Mi	Liver	60'	1 11	1 16	
Pa	Liver	60'	0 90	0 78	
Gr	L pectoralis major	30'	1 55	1 49	
Jo	L pectoralis major	30'	2 07	2 04	
St	L pectoralis major	30'	2 90	2 91	
Gr	L gastrocnemius	120'	0 89	0 79	
P	L gastrocnemius	30	1 01	0 77	
W	R gastrocnemius	30' and 60'	2 18	2 56	2 16

Gastrocnemius   In 11 normal subjects the mean muscle glycogen was 1 30% (range 0 78-2 19), (Table IV)   In every case the histology of gastrocnemius or pectoralis major was normal

(b) *Diabetes*   The mean muscle glycogen in the pectoralis major (1 59%) in 6 diabetics was lower than in normal subjects, but there was no statistically significant difference between the two groups   The muscles in diabetes were histologically normal (Table 5)

TABLE V

Muscle glycogen in diabetes
(*In all these cases the muscle sampled was pectoralis major*)

Case	Sex and age	Blood glucose m /100 ml	Alkali reserve vol /100 ml	Blood lactic acid mg /100 ml	Muscle glycogen g per 100 g
J T	M 67	260	59	4 0	2 49
W W	M 46	180	—	8 5	2 02
R C	F 62	300	57	12 0	2 03
G W	M 32	248	36	—	1 18
H B	F 32	258	46	7 0	1 12
M D	M 24	264	49	5 0	0 72
				Mean	1 59

(c) *Acute hepatitis*   In three cases the muscles showed a normal glycogen content and were histologically normal (Table 6)

TABLE VI

Muscle glycogen in acute hepatitis

Case	Sex and age	Serum bilirubin mg /100 ml	Muscle glycogen g per 100 g
C P	M 54	4 2	1 43
W P	M 53	15 5	1 65
J S	M 47	9 5	2 02

Distribution of muscle glycogen   In three instances simultaneous samples were taken from right and left pectoralis major or gastrocnemius There was little difference in the glycogen content from the two sides (Table VII)

In three instances, comparison of pectoralis major and gastrocnemius showed a higher glycogen value for the former (Table VIII)

TABLE VII

Comparison of the glycogen contents of muscles from the right and left side

Case	Muscle	Glycogen g per 100 g	
		Right	Left
G	Pectoralis major	1 26	1 17
St	Gastrocnemius	2 02	2 19
I G	Pectoralis major	1 22	1 19

The trauma of repeated sampling is said to lower the muscle glycogen In six normal subjects repeated muscle biopsies were done at intervals to determine if this effect could be minimised by careful sampling away from the site of previous biopsy (Table III) In four cases the difference between the samples was small In one there was an appreciable fall and in one case where three biopsies were taken at 30-minute intervals, the second sample was higher than the first and the third almost the same as the first We considered that with careful sampling an appreciable fall in muscle glycogen could be attributed to something other than trauma in the experiments reported in Part II (6) especially if the value rose again at the end of the experiment to the initial level

TABLE VIII

Comparison of glycogen content of pectoralis major and ca trocnemius

Case	Glycogen g per 100 g	
	Pectoralis major	Gastrocnemius
Jo	2 07	1 10
St	2 90	2-02
Bo	1 59	1 14

The glycogen content of 10 normal human livers biopsied at operation was 1 21-6 31 (mean 3 15%) (9) The total carbohydrate content of livers from subjects dying suddenly was 1 56-6 17% (13) In the present group of hospital patients the mean hepatic glycogen has proved lower although we agree with other workers in finding a very wide range

In animals from which the pancreas has been removed, hepatic glycogen is very low, (11, 3), but is restored with insulin therapy (3) In fatal cases of human diabetes total hepatic carbohydrate is variable but not constantly low (13) Serial aspiration liver biopsies in a patient with diabetic coma have shown very low hepatic glycogen, improving with treatment (1) Similar biopsies in two cases of severe juvenile diabetes showed reasonably normal values (8) The glycogen in all these aspiration biopsies, however, was only approximately determined on sections stained for glycogen In the present series the mean hepatic glycogen in diabetes, although diminished, was not as low as had been expected The difference between the means for diabetic and normal subjects was not statistically significant and the overlap between the two groups was very great The hepatic glycogen values did not correlate with the clinical type of diabetes, the fasting blood sugar or the degree of ketosis Under basal conditions hepatic glycogen altered but little over one hour and is little affected by the trauma of a preceding biopsy

Muscle glycogen Although duplicate analyses were not so consistent as for liver biopsies, provided care is taken in the selection of samples results seem reliable Sections for histology were always cut from part of the muscle adjoining that taken for glycogen analysis The histological picture is reasonably uniform, the muscle bundles being separated by only a minimum of fibrous tissue

Only one report has been found of human muscle glycogen Limbs amputated under chloroform anaesthesia showed much lower values than those of the present series (12) Distally placed muscles contain less glycogen than proximal ones This also proved the case when we compared the glycogen of pectoralis major with that of gastrocnemius The high glycogen content of muscle, sometimes higher than that of liver, was quite unexpected Even assuming variability from muscle to muscle the large muscular mass in the body must provide a great carbohydrate reserve This glycogen, moreover, is readily mobilised both by adrenaline (6), and, as unpublished observations have shown, to an even greater extent during a rigor

In animals after removal of the pancreas muscle glycogen is low (11), though not so low as that of the liver (3) There are apparently no reports of muscle glycogen in human diabetes In our six patients, although the mean muscle glycogen was less than that in normal subjects, it was still well within the normal range

LIVER AND MUSCLE GLYCOGEN IN NORMAL SUBJECTS, IN DIABETES MELLITUS AND IN ACUTE HEPATITIS

PART II THE EFFECTS OF INTRAVENOUS ADRENALINE

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Much has been written on the effects of adrenaline on carbohydrate metabolism in animals   In man, however the changes produced in muscle and hepatic glycogen do not seem to have been studied   In human diabetes the glycemic response to adrenaline is diminished (1, 17)   It seemed important to repeat these observations, and at the same time to measure the changes in liver and muscle glycogen, in blood lactic acid and in serum potassium   Results have been compared both with those found in normal subjects and in patients with acute hepatitis in whom a poor glycemic response to adrenaline has also been reported (3 17)

The patients with acute hepatitis were graded on the basis of hepatic histology and on serum bilirubin and protein changes (23) Two were of a severe and one of a mild grade

Experimental procedure Observations were made with the subject previously confined to bed and without food for twelve hours Sodium amytal 0 2 g by mouth was sometimes given 30 minutes previously This mild sedative was not found to affect the results

Adrenaline was infused into a forearm vein for approximately one hour, preceded and followed by control periods also of one hour During the entire three hours a record was kept of brachial blood pressure and pulse rate At fifteen minute intervals capillary and venous blood samples were taken from the opposite arm for the estimation of blood glucose, lactic acid and serum potassium concentrations In some subjects changes in hepatic glycogen were determined by aspiration liver biopsies before and during the adrenaline infusion In others, muscle glycogen changes were followed by sampling before, during and after the adrenaline In some instances urine obtained by catheter before and after the infusion was analysed for sugar and lactic acid Oxygen consumption was also determined before, during and after the adrenaline

Adrenaline preparation and dosage The same preparation of adrenaline was used throughout (Allen and Hanbury, 1 1,000) The 0 5 ml ampoules were stored in the dark at 4° C The diluted intravenous solution was prepared immediately before use, 1 ml of the 1 1,000 solution being added to 500 ml normal saline acidified with 250 mg ascorbic acid The diluted solution was given in a dose of 0 067-0 11 µg per kg per minute from a graduated burette with drip attachment The infusion rate was reasonably steady, 10-13 ml being given in every five minutes

Muscle and hepatic biopsies The technique has been described previously (12)

Venous blood samples A venous tourniquet was placed round the wrist to exclude blood from the hand The samples were then taken from an antecubital vein with the minimum of stasis, and analysed for blood lactic acid (18), and serum potassium (16)

Capillary blood samples were taken in 0 05 ml pipettes from a freely bleeding ear prick Duplicate samples were taken on each occasion, and were analysed for glucose (11)

Oxygen consumption In the earlier observations a ten minute collection of expired air was taken in a Douglas bag and the oxygen and carbon dioxide content determined with the Haldane gas apparatus Later it was realised that sufficiently accurate values could be obtained with the Benedict-Roth spirometer

TABLE II

Diabetes and acute hepatitis

The effect of intravenous adrenaline on blood sugar, blood lactic acid and serum potassium, muscle glycogen and hepatic glycogen concentration

Muscle glycogen values refer to pectoralis major

Case No	Dose µg per kg min	Blood sugar mg per 100 ml								Blood lactic acid mg per 100 ml						Serum potassium mg per 100 ml		Muscle glycogen g per 100 g				Liver glycogen g per 100 g	
		\ Time after start of adrenaline (min)						Max rise	Max fall	Time after start of adrenaline (min)					Max rise	Init ial	Max fall	Time after start of adrenaline (min)				Time after start of adrenaline (min)	
		0	15	30	40	60	190			0	15	30	60	120				0	30	60	120	0	60
Diabetes																							
D 1	0 071	240	257	303	318	287	208	+78	0	57	63	62	156	61	98	187	15	—	—	—	—	0	111
D 2	0 084	188	208	310	342	357	218	+69	0	75	134	131	104	49	117	183	33	203	164	152	180	—	—
D 3	0 083	309	308	320	313	365	375	+56	— 1	44	08	91	08	46	47	204	07	—	—	—	—	—	—
D 4	0 082	300	295	307	311	352	352	+52	— 6	90	114	300	296	96	210	101	30	303	101	140	193	230	—
D 5	0 073	201	250	286	290	315	278	+61	65	55	130	138	30	39	83	180	10	073	013	036	055	179°	—
D 6	0 083	353	263	273	278	296	206	+25	73	126	136	100	67	62	93	210	30	113	075	093	156	215°	—
D 7	0 075	218	217	230	204	280	260	+10	— 1	—	—	—	39	—	—	180	20	118	087	066	119	—	—
D 8	0 10	268	210	239	266	292	232	+ 8	— 19	90	100	93	57	46	80	175	25	—	—	—	—	—	—
D 9	0 075	266	206	238	280	248	248	+10	— 20	180	183	130	117	58	138	205	30	—	—	—	—	—	—
D 10	0 070	206	196	173	190	199	199	+ 0	— 73	103	126	107	58	85	—	—	—	—	090	066	085	101	—
D 11	0 085	173	118	168	180	210	210	+ 7	— 34	138	140	67	30	30	170	20	118	085	090	256	88	575
D 12	0·085	258	209	210	200	200	200	+ 0	— 26	04	03	07	03	06	68	200	20	249	—	266	208	—	298°
D 13	0 072	211	104	194	100	100	100	+ 4	— 18	101	88	85	03	33	210	20	085	—	103°	256°	—
Acute hepatitis																							
H 1	0 070	171	117	119	160	113	+73	0	138	205	203	287	145	110	103	— 1	—	—	118	114	—	—	—
H 2	0·082	89	88	107	111	93	+22	— 1	67	90	94	90	70	178	20	165	121	110	0·06	11°	—	—
H 3	0 053	91	60	0	103	70	+17	— 5	101	107	106	100	53	200	30	203	—	—	—	—	—	—

° Under basal conditions on day following adrenaline infusion

Results

Results are tabulated (Tables 1-4) and examples of the response in normal subjects, in diabetes and in acute hepatitis are illustrated (Figs 1-3)

General effects of the intravenous adrenaline infusion

There were few symptoms although sometimes the adrenaline produced headache or a feeling of light-headedness Facial pallor was a constant feature It persisted throughout the infusion Within two minutes of stopping the adrenaline a facial flush was noticed which lasted for about five minutes

TABLE III

Diabetes

The effect of adrenaline on urinary sugar

Case No	Urinary sugar g 1 hour excretions	
	Before adrenaline	During adrenaline
D 2	1 5	2 5
D 3	2 3	1 6
D 6	3 0	4 0
D 12	2 0	2 5

(b) *Diabetes* Five of the thirteen patients studied (D 1-5, Table 2) showed an essentially normal increment in blood sugar In these five subjects the return to normal was slow and in some the level continued to rise for as long as thirty minutes after stopping the adrenaline In all five, the blood sugar an hour after the conclusion of the infusion was still raised above the resting value

Eight of the patients (D 6-13, Table 2) showed a diminished glycæmic response to adrenaline, the maximum rise in blood sugar being less than 25 mg per 100 ml   The blood sugar did not increase steadily during the

TABLE IV

Oxygen consumption before, during and after adrenaline infusion

Case No	Dose of adrenaline μg per kg per min	Oxygen consumption c c per min			Increase in O_2 consumption c c per min
		Basal	During adrenaline	1 hr after	
Normal subjects					
N 1	0 087	165	203	160	38
N 4	0 084	198	222	209	24
N 6	0 081	224	257	222	33
N 10	0 077	175	235	198	60
N 12	0 088	170	240	—	70
N 14	0 080	189	202	184	13
N 16	0 077	154	176	154	22
				Mean	37
Diabetics					
D 4	0 082	160	214	177	54
D 6	0 083	202	252	223	50
D 7	0 075	264	309	285	45
D 9	0 075	251	301	249	50
D 10	0 079	185	220	195	35
D 11	0 085	196	237	164	41
D 12	0 082	233	255	237	22
D 13	0 072	133	151	—	18
				Mean	39
Acute hepatitis					
H 1	0 079	234	281	243	47

infusion as in the normal subjects   In fact in all but three of the diabetics at the beginning of the adrenaline infusion there was a fall in blood sugar, in some cases quite pronounced   The blood sugar usually returned to the fasting level or above it by the end of 60 minutes infusion   In three cases there was a sharp increase in blood sugar when the adrenaline infusion was stopped
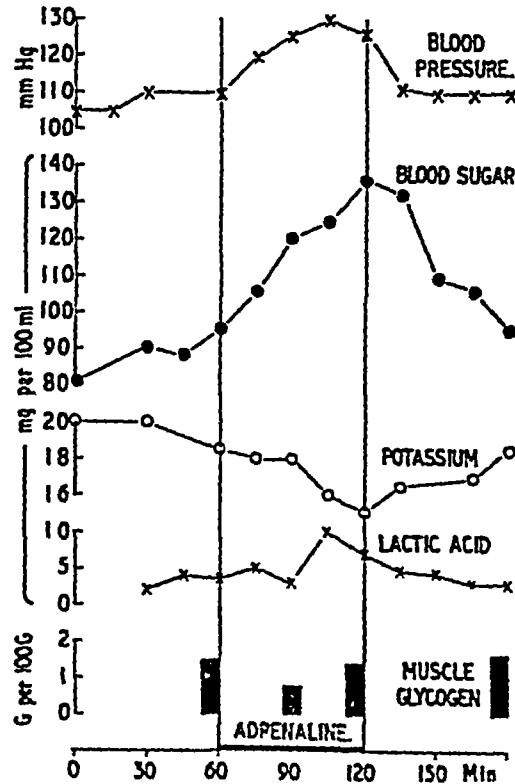
Blood lactic acid

(a) *Normal subjects* Adrenaline caused an increase in blood lactic acid, the maximum being 2 1-17 0 mg per 100 ml above the fasting value Again there was a rough correlation with dosage The maximum value was recorded at varying times after starting the adrenaline In eight it occurred sixty minutes later, in seven forty-five minutes later, and in one only thirty minutes after beginning the infusion An early peak value was associated with a falling off of blood lactic acid towards the end of the
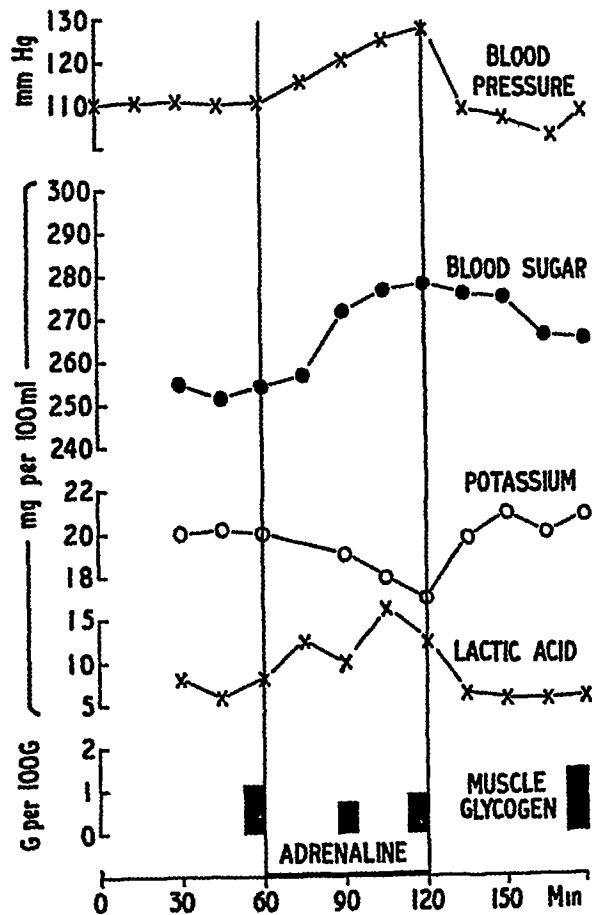

Fig 2   *Diabetes*   D 6   Female, age 32   The effect of intravenous adrenaline on systolic blood pressure, blood sugar, serum potassium, blood lactic acid and muscle glycogen   Dose of adrenaline 0 083 µg per kg per min

infusion despite continuance of the adrenaline and continued rise of blood sugar   In fifteen of the sixteen subjects the blood lactic acid was back to the initial value within forty-five minutes of stopping the infusion

(b) *Diabetes* Adrenaline constantly caused an increase in blood lactic acid similar in degree to that in normals   The maximum increment of 4 8-21 0 mg per 100 ml was often reached before the conclusion of the infusion On stopping the infusion the blood lactic acid rapidly returned to normal

(c) *Acute hepatitis*   The three patients also showed an increase in blood lactic acid similar to that in normal subjects

Serum potassium

(*a*) *Normal subjects*   The level fell, the lowest value being reached at the conclusion of the adrenaline infusion   It was 1 0-5 8 mg /100 ml below the fasting value   The subsequent return to normal was slow and in five subjects the level was still reduced an hour after the conclusion of the adrenaline infusion

(*b*) *Diabetes*   The level fell to 1 0-3 9 mg /100 ml below the initial value, the depression being slightly less than in normals

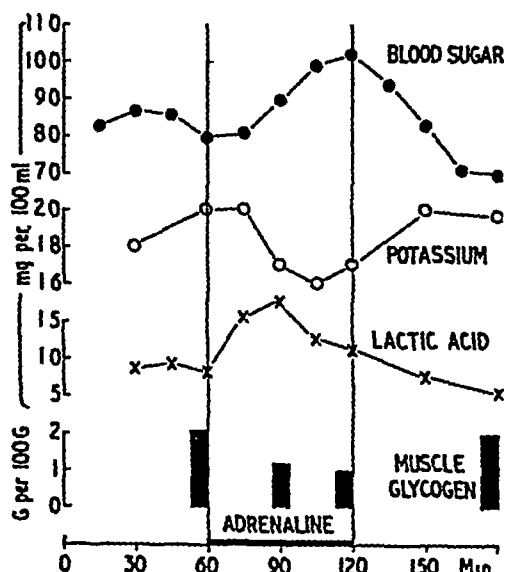(c) *Acute hepatitis*   All three patients showed a fall in serum potassium similar to that seen in diabetes

I ig 3   *Iente hepatiti* H 3   Male age 47   The effects of intravenous adrenaline on capillary blood sugar serum potassium, blood lactic acid and muscle glycogen   Dose of adrenalin 0 053 µg per kg per min

Muscle glycogen

(*a*) *Normal subjects*   In all subjects during the first thirty minutes of the adrenaline infusion the muscle glycogen diminished   During the second thirty minutes there was a variable change , three patients showed a further depletion of muscle glycogen but in the other six the level rose   It seemed possible that the maximum depression of muscle glycogen might occur earlier than thirty minutes after beginning adrenaline   Samples were therefore taken both fifteen and thirty minutes after the start of the infusion (Cases N 4 (2) and N 5) (Table I)   Although some depletion had occurred at fifteen minutes the muscle glycogen showed a lower value at thirty

minutes In only six subjects were muscle glycogen values estimated one hour after the conclusion of the adrenaline infusion In four of these glycogen exceeded or was within 0 1 g per cent of the fasting value In two it was still below the initial figure and in one of these the level was even below that found at the conclusion of the adrenaline A sampling error is probable in this instance (Case N 5, Table I)

In all the observations except one, pectoralis major was used for sampling In Case N 4, Table I, however, pectoralis major and gastrocnemius were used on separate occasions The leg muscle showed the lower initial glycogen values and the depletion during the adrenaline infusion was less than that previously recorded in pectoralis major in this subject

(b) *Diabetes* Changes in muscle glycogen were more consistent In all the six patients studied, during the first thirty minutes of the adrenaline infusion the muscle glycogen diminished Unlike the normal group, in four of the subjects at the end of the hour the value was lower or within 0 1 g of that recorded at thirty minutes In the other two, the one hour reading was greater than that found at thirty minutes

In all six patients the muscle glycogen increased during the hour after the conclusion of the adrenaline infusion In four it was within 0 1 g and in two within 0 22 g , of the initial fasting value

Owing to the lower initial values in diabetes the depletion of muscle glycogen as a result of adrenaline, expressed as a percentage change, is more conspicuous than in normal subjects

(c) *Acute hepatitis* In both the patients with acute hepatitis (severe) there was a depletion of muscle glycogen both at thirty and at sixty minutes after the start of the infusion Recovery to pre-adrenaline levels had occurred one hour later, thus reacting similar to the diabetics

Tables I and II also bring out the fact that, in all the groups studied, the higher the initial muscle glycogen level the greater the depletion produced by adrenaline

Liver glycogen

For various reasons two liver biopsies could be performed on only a few subjects Samples were taken at the end of the control period and just before the adrenaline was stopped

(a) *Normal subjects* In all five subjects hepatic glycogen diminished by 0 26-0 92 g per cent On this very small series there appeared to be little relation between the fasting hepatic glycogen and the response to adrenaline

(b) *Diabetes* Two biopsies were performed in only three subjects In one (Case D 1, Table II) adrenaline caused a depletion of hepatic glycogen This subject was one of the elderly insulin-insensitive diabetics whose

response to adrenaline was in every other way normal   In the other two adrenaline caused an increase in hepatic glycogen   These two patients were among those that gave a poor glycæmic response and the diabetes was of the acute variety   In four subjects on the day succeeding the infusion the fasting hepatic glycogen was measured under basal conditions   Two had given a normal and two a small blood sugar rise with adrenaline   In each case hepatic glycogen was within the normal range

(c) *Acute hepatitis*   The hepatic lesion is patchy and analysis of one small sample must be only a poor indication of hepatic glycogen as a whole   In one subject, who gave a poor blood glucose increase with adrenaline   the value under basal conditions, was low

Urinary sugar and lactic acid

(a) *Normal subjects*   In five of the subjects urine passed immediately after the period of the adrenaline infusion did not contain sugar or lactic acid

(b) *Diabetes*   In four subjects, urine samples passed during the hour preceding the adrenaline and just after its conclusion were tested for sugar (Table III)   In three there was an increased urinary sugar during the adrenaline infusion   In one there was an apparent diminution   The amount of sugar excreted was not related to the extent of the glycemic response to adrenaline

Oxygen consumption (*Table IV*)

(a) *Normal subjects*   In seven normal subjects adrenaline increased the oxygen consumption by 22-70 ml per min   With one exception the oxygen consumption one hour later had returned to the basal value

(b) *Diabetes*   In eight diabetics adrenaline increased the oxygen consumption by 22-54 ml per min   There was little difference between these results and those for normal subjects   In five patients the oxygen consumption one hour later had returned to within 10 ml per min of the basal value   In another two the oxygen consumption was still increased by 21 and 17 ml per min

(c) *Acute hepatitis*   Oxygen consumption was measured in only the patient with the mild hepatitis   The increase was normal and the value had one hour later returned to within 9 ml per min of the resting value

The effect of intravenous adrenaline on an artificially raised blood sugar in normal subjects

It was calculated that in an average sized man 20 grammes of glucose intravenously would be required to raise the blood sugar 100 mg per 100 ml and that maintenance of this increase over a period of hours would require an infusion of about 0 5 g glucose per minute

In three normal men, after a preliminary control period of one hour, a priming infusion of 50 ml of 50 per cent glucose was given slowly into a forearm vein　During the next three hours 10 per cent glucose solution was infused at the rate of about 5 ml a minute　The blood sugar increased to about 200-300 mg per 100 ml and for the next hour remained surprisingly constant　During the second hour adrenaline was also infused, into another forearm vein　During the third hour glucose alone was given

Results are shown in Table V and an example is illustrated (Fig 4) It is seen that the adrenaline increased the blood sugar 26-63 mg per 100 ml above the level reached with glucose alone　In the third hour the blood sugar returned to the pre-adrenaline level

TABLE V

Normal subjects　The effect of adrenaline on an artificially raised blood sugar

Subject	Sex and age		Weight kg	Dose adrenaline μg per kg per min	Blood sugar mg per 100 ml			
					Initial	1st hour* glucose alone	2nd hour* glucose + adrenaline	3rd hour* glucose alone
R K	M	41	55	0 083	98	214	240	204
W M	M	25	57	0 073	96	222	285	237
T S	M	19	53	0 092	103	351	390	348

* Blood sugar values are the mean of the four readings obtained during the hour

DISCUSSION

The glycogen content of small samples of liver and muscle has been taken as representative of total liver and muscle glycogen (12)　The source of error arising from the size of the samples is inevitably greater in human than in animal observations　Nevertheless, the changes measured in normal subjects are in general agreement with opinion based on animal work　It therefore seems probable that the departures from normality found in acute diabetics are of significance

The effects of intravenous adrenaline in normal subjects

It has been shown here, and by others (6, 4, 15), that in normal subjects intravenous infusion of adrenaline produces a rise in blood sugar, blood lactic acid and oxygen consumption, and a fall in serum potassium, the muscle glycogen at first falls and later tends to return to its original level, the liver glycogen falls　It would seem, therefore, that glycogen has been mobilized from liver and muscle　In animals, observations of a generally similar character have been reported (9, 10, 21) though the fall in liver glycogen may be followed by a return to normal or above (7, 8, 22) (Fig 5), and a return to normal of muscle glycogen has not been observed (2), species and dose may account for these differences

Table VI shows an attempt to strike a balance using values before and after 60 minutes of adrenaline infusion   Forty-seven of the 59 grammes of carbohydrate mobilized from liver and muscle can be accounted for   The discrepancy may have several explanations, namely (a) that the biopsies do not fairly represent the total hepatic and muscle glycogen, (b) that sources
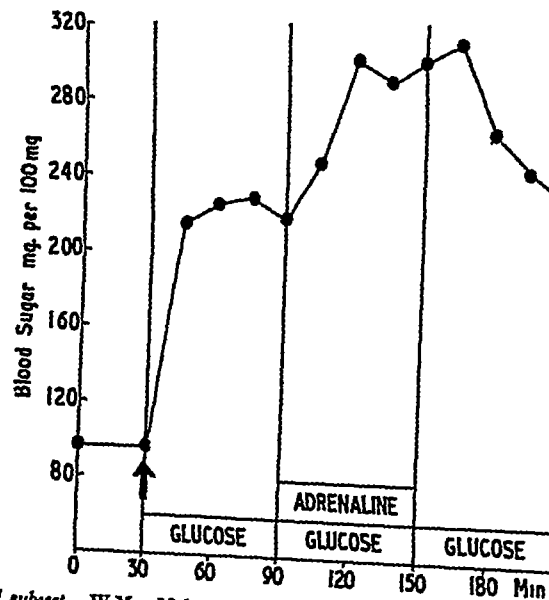

Fig 4   *Normal subject*   W M   *Male, age 25*   The effect of intravenous adrenaline on blood sugar already raised by intravenous glucose   25 g glucose was given as a priming dose indicated by the arrow   For the next three hours the amounts of glucose infused per hour were 31 5 g , 30 8 g and 30 3 g   Dose of adrenaline — 0 073 μg per kg per min
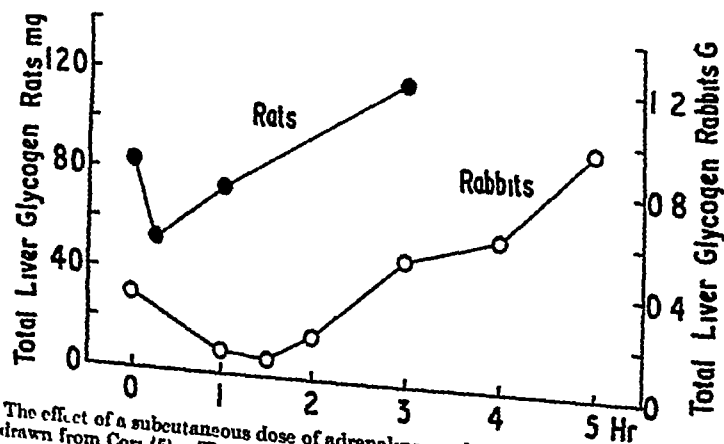
Fig 5   The effect of a subcutaneous dose of adrenaline on the liver glycogen of rats and rabbits. Re drawn from Cori (5)   The dose of adrenaline given to the rats was 0 02 mg per 100 g body weight   This dose was not glycosuric   Dose of adrenaline given to rabbits was 1 mg for a 2 kg animal   The same authors (22) also found depletion of liver glycogen at 11 hours with a dose of 0 1 mg per kg , which dose did not produce glycosuria

of energy other than carbohydrate are used during the infusion, and (c) that the rises in concentration of glucose and lactic acid are not uniform throughout the various compartments of the body water during the infusion, for example, the glucose concentration in the cells may not increase to the same extent as in the extra cellular fluid (5)   We cannot exclude a transfer of carbohydrate to fat   Although in rats adrenaline is said not to influence glycogen deposition in fat depots (26) observations with Deuterium have shown that, in the presence of a carbohydrate plethora, large quantities of glucose are used to synthesize fatty acids (25)

<div align="center">

TABLE VI

Carbohydrate balance after adrenaline infusion for 60 min in normal subjects

</div>

Decrease in liver glycogen 0 40%	Increase in blood sugar 48 mg per 100 ml
Glucose liberated $\dfrac{0\ 49 \times 1,000}{100} \times 1\ 5 = 7\ 3\ g$	Total increase of body sugar $= 24\ 8\ g$
Decrease in muscle glycogen 0 27% Carbohydrate liberated from muscle $\dfrac{0\ 27 \times 1,000}{100} \times 19 = 51\ 3\ g$	Increase in blood lactic 8 7 mg per 100 ml acid Total increase in body lactic acid $=$ 4 5 g O. consumption 220 ml per min
Total carbohydrate liberated 58 6 g	Carbohydrate combustion $\dfrac{60 \times 220}{750} = 17\ 0\ g$
	Total carbohydrate accounted for $=$ 46 9 g

The changes are calculated from the mean values of 20 observations (Table I)   Mean weight of subjects is 56 kg   Muscle mass is calculated as 33% of body weight = 19 kg   Liver mass is calculated as 0 0267 kg per kg body weight = 1 50 kg   Total body water is taken as 70% of body weight = 40 litres   Blood plasma is 92%, and blood cells 60% water   Assuming the haemotocrit to be 45%, whole blood is 77 6% water and, therefore, an increase in blood sugar of 48 mg per 100 ml whole blood is equivalent to an increase of 62 mg per 100 ml blood water The total increase of glucose in the body is therefore 24 8 g   Similarly, the total increase in body lactic acid is calculated as 4 5 g   It is assumed that carbohydrate only is catabolized during the test period and that 1 g of glucose requires 750 c c O. for its utilization

The effect of intravenous adrenaline in diabetes and acute hepatitis

In five diabetics the rise in blood sugar and lactic acid induced by adrenaline infusion was of the normal size , in one of these patients liver biopsy showed a fall in glycogen , in three, muscle biopsy showed muscle glycogen to behave in an essentially normal fashion, though the fall was of longer duration than in the normal subjects

In the remaining eight diabetics, however, adrenaline produced only a small rise in blood sugar, and in seven of these there was indeed a fall at the beginning ranging from 12 to 34 mg per 100 ml , muscle glycogen behaved as in the other diabetics , liver glycogen rose in the two cases where it was measured, in one to nearly twice the original value

The smallness of this rise in blood sugar might be due to increased peripheral utilization or to diminished glycogen mobilization from liver (19)   During adrenaline infusion glucose is not excreted in the urine by diabetics to an extent that would affect the blood sugar (Table III)   Nor is increased utilization the cause (14), for the increase in oxygen consumption induced by adrenaline is no greater than normal in the diabetics (Table IV) Accelerated removal of glucose by a high blood concentration is not apparently the cause, for the two patients with the highest fasting blood levels, both showed a normal rise , moreover, adrenaline has been shown to produce normal responses in normal subjects whose blood sugar has been artificially raised by a constant glucose infusion (Fig 4)

This brings us to consider the remaining possibility that the impaired response to adrenaline is due to deficient release of glucose from the liver It has been suggested that hepatic glycogen is low in human diabetes (20) and this would explain the smallness of the glycæmic response to adrenaline However, we have found that liver glycogen in diabetics, although slightly lower on the average than in normal subjects, is not diminished to an extent likely to account for the poor glycæmic response   Two of the diabetics (D 11 and D 13) showing only a small rise in blood sugar had liver glycogen values above the mean for normals   Also two of the normal subjects (N 2 and N 6) with a normal blood sugar rise had liver glycogen values below the mean for diabetics

Thus it seems unlikely that the poor glycæmic response in diabetics is due to an inadequate quantity of liver glycogen   It seems more probable that the diminished response is due to an inadequate mobilization of liver glycogen by adrenaline, but we do not regard our findings as proof of this hypothesis

It is known that in parenchymal liver disease, adrenaline fails to produce a normal rise in blood sugar (3, 17)   In these cases it is possible that the level of liver glycogen is low   The patchy nature of the lesion in acute hepatitis makes it difficult to assess the total liver glycogen from a small biopsy sample

The relation of the adrenaline response to the type of diabetes

Four of the five diabetics with a normal glycæmic response to adrenaline were of the "stable insulin-insensitive" type , the fifth was a young man with acute diabetes, in ketosis, and clearly of the "juvenile" type   Five of the seven diabetics showing a decreased glycæmic response were of the young acute   insulin-sensitive ' type of diabetes , the other two, although older were also of acute onset, had no degenerative complications and would probably be classed with this group clinically

Thus it seems that a small glycæmic response to adrenaline is usually associated with the " juvenile insulin-sensitive " group of diabetics

The insulin insensitivity of some diabetics has been related to defective hepatic function and particularly to fatty change in the liver (24) Our findings do not support this Hepatic histology in all the diabetics studied was within normal limits

SUMMARY

1   Intravenous adrenaline in a dose of 0 058-0 11 μg per kg per minute was given for one hour to 19 normal subjects, 13 diabetics and 3 patients with acute hepatitis Changes in hepatic and muscle glycogen, blood sugar, blood lactic acid and serum potassium, and in oxygen consumption were determined

2   In normal subjects adrenaline caused a rise in blood sugar and lactic acid, and a fall in serum potassium There was no glycosuria Oxygen consumption increased Muscle glycogen fell during the first thirty minutes of the infusion, but tended to recover during the second thirty minutes One hour after the infusion the muscle glycogen was usually normal There was a constant fall of hepatic glycogen at the end of the hour of adrenaline infusion

3   At the conclusion of the adrenaline infusion we could account for 47 of the 59 g carbohydrate mobilized from liver and muscle This discrepancy may well be due to the sample of muscle being not truly representative of muscle as a whole with regard to its glycogen content

4   Five diabetics gave a normal response to intravenous adrenaline The remaining eight showed only a small rise in blood sugar Rise in lactic acid and in oxygen consumption were normal In six diabetics adrenaline caused a depletion of muscle glycogen during the hour of the infusion , muscle glycogen was restored to normal one hour later In two diabetics who showed a small rise in blood sugar adrenaline increased hepatic glycogen

5   Two patients with severe acute hepatitis gave a subnormal glycaemic response to adrenaline Changes in blood lactic acid and muscle glycogen were similar to those seen in normal subjects

6   Normal subjects with an artificially raised blood sugar showed a further increase in blood sugar when given adrenaline

7   Possible factors to explain the decreased glycaemic action of adrenaline in diabetes and in acute hepatitis are discussed

8   The majority of diabetic patients with the poor glycaemic response to adrenaline were of the juvenile unstable type The patients reacting normally were usually of the elderly stable type

APPENDIX I

S I   — soluble insulin
Z P I — zinc protamine insulin
C H O — carbohydrate
G   — Gerhardt's urinary ferric chloride test *
R   — Rothera's urinary nitroprusside test *
A R   — alkali reserve Vol /100 ml *
       * These tests refer to the day of the adrenaline infusion

Normal subjects

N					
N 1	E H	Male, aged 57	Ht 166 cm	Wt 53 kg	Convalescent duodenal ulcer
N 2	V H	Male, aged 44	Ht 173 cm	Wt 55 kg	Convalescent pneumonia
N 3	R C	Male, aged 42	Ht 166 cm	Wt 53 kg	Convalescent duodenal ulcer
N 4	C B	Male, aged 47	Ht 178 cm	Wt 54 kg	Convalescent duodenal ulcer
N 5	F S	Female, aged 70	Ht 616 cm	Wt 52.5 kg	Arthritis
N 6	T L	Male, aged 24	Ht 176 cm	Wt 65 kg	Idiopathic epilepsy
N 7	R K	Male, aged 41	Ht 158 cm	Wt 55 kg	Convalescent pneumonia
N 8	R N	Male, aged 46	Ht 159 cm	Wt 48 kg	Convalescent gastric ulcer
N 9	H G	Male, aged 63	Ht 176 cm	Wt 66 kg	Neurosyphilis
N 10	A T	Male, aged 40	Ht 181 cm	Wt 63 kg	Convalescent duodenal ulcer
N 11	R W	Male, aged 30	Ht 164 cm	Wt 65 kg	Dyspepsia
N 12	A C	Male, aged 56	Ht 171 cm	Wt 46 kg	Depressive psychosis
N 13	J B	Male, aged 73	Ht 162 cm	Wt 49 kg	Convalescent bronchitis
N 14	J G	Male, aged 65	Ht 181 cm	Wt 68 cm	Convalescent gastric ulcer
N 15	R P	Male, aged 55	Ht 173 cm	Wt 51 kg	Convalescent gastric ulcer
N 16	L B	Female, aged 21	Ht 158 cm	Wt 40 kg	Chronic postencephalitic paraplegia
N 17	M H	Female, aged 67	Ht 161 cm	Wt 46 kg	Chronic bulbar palsy
N 18	J T	Male, aged 20	Ht 176 cm	Wt 61 kg	Convalescent tonsillitis
N 19	J O	Male, aged 66	Ht 168 cm	Wt 67 kg	Carcinoma of colon Hb 81%

Patients with diabetes

D 10   A G   Male, aged 25   Ht 179 cm   Wt 66 kg   Sudden onset 6 weeks ago with thirst, polyuria and weight loss   B P 115/80   No S I for 2 days   G —   R — Finally stabilized on 2522 C , 287 g , C H O and 30 units Z P I , 20 units S I daily

D 11   A W   Female, aged 60   Ht 166 cm   Wt 55 kg   Sudden onset 6 months ago with polyuria, thirst and weight loss   Herpes Zoster   No Z P I for 5 days   G — R tr   A R 55 vol /100 ml   Finally stabilized on 2170 C , 262 g , C H O and 28 units Z P I   12 units S I daily

D 12   J T   Male, aged 67   Ht 176 cm   Wt 52 kg   Sudden onset 6 weeks ago with thirst and 2 stone weight loss   B P 122/75   Never had insulin   G — R +   A R 59 vol /100 ml   Finally stabilized on 1965 C , 205 g , C H O and 25 units Z P I   10 units S I daily

D 13   Z H   Female, aged 59   Ht 173 cm   Wt 58 kg   14 months previously operation biopsy showed chronic pancreatitis   12 months later symptomless glycosuria   86% fat absorption   B P 125/75   Never had insulin   G —   R —   Finally stabilized on 1730 C , 25 g , C H O and 10 units S I b i d   Has gained 7 kg in 4 months

Acute hepatitis

H 1   C B   Male, aged 54   Ht 177 cm   Wt 73 kg   Serum hepatitis   Jaundiced 6 days   Urobilin present in urine   S bilirubin 4 2 mg, S albumin 3 4, S globulin 3 7 g all per 100 ml

H 2   W P   Male, aged 53   Ht 176 cm   Wt 76 kg   Infective hepatitis   Jaundiced 14 days   Urobilin absent from urine   S bilirubin 16 5 mg, S albumin 3 2 g, S globulin 2 6 g , all per 100 ml

H 3   J S   Male, aged 47   Ht 165 cm   Wt 50 kg   Post transfusion hepatitis   Jaundiced 12 days   Urobilin absent from urine   S bilirubin 9 5 mg, S albumin 3 1 g, S globulin 3 0 g , all per 100 ml

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